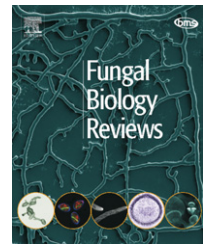




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Review

Wood decay under the microscope[☆]

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ABSTRACT

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Many aspects of the interactions between host wood structure and fungal activity can be revealed by high resolution light microscopy, and this technique has provided much of the information discussed here. A wide range of different types of decay can result from permutations of host species, fungal species and conditions within wood. Within this spectrum, three main types are commonly recognised: brown rot, white rot and soft rot. The present review explores parts of the range of variation that each of these encompasses and emphasizes that degradation modes appear to reflect a co-evolutionary adaptation of decay fungi to different wood species or the lignin composition within more primitive and advanced wood cell types. One objective of this review is to provide evidence that the terms brown rot, white rot and soft rot may not be obsolete, but rigid definitions for fungi that are placed into these categories may be less appropriate than thought previously. Detailed knowledge of decomposition processes does not only aid prognosis of decay development in living trees for hazard assessment but also allows the identification of wood decay fungi that can be used for biotechnology processes in the wood industry. In contrast to bacteria or commercial enzymes, hyphae can completely ramify through solid wood. In this review evidence is provided that wood decay fungi can effectively induce permeability changes in gymnospermous heartwood or can be applied to facilitate the identification of tree rings in diffuse porous wood of angiosperms. The specificity of their enzymes and the mild conditions under which degradation proceeds is partly detrimental for trees, but also make wood decay fungi potentially efficient biotechnological tools.

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1. Introduction

In natural ecosystems, there is a dynamic equilibrium between the accumulation of woody biomass and its breakdown. In this way, a permanent cover of trees or shrubs is maintained, while the carbon and minerals that they have

fixed are recycled. At the same time the survival of a wide range of woodland plants and animals is fuelled by the energy released in the breakdown of wood. Decay fungi play a major role in the processes of decomposition since, alone among microorganisms, they have evolved the means to decompose large volumes of wood completely.

[☆] Dedicated to Alex L. Shigo.

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The balance between trees and decay fungi represents the state of play in a co-evolutionary battle, which has lasted for hundreds of millions of years, and in which wood has been the main prize. The success of trees as a dominant form of land vegetation has depended on their being able to maintain a perennating woody structure, which is their means of attaining both height and longevity. This defensive strategy protects the woody cylinder against loss of integrity of both its water-conductivity and its mechanical properties.

Decay in standing trees is of major concern in relation to human safety, since it often weakens stems, branches or roots enough to increase the chance of mechanical failure. Small volumes of decay commonly occur in trees without causing any mechanical weakness, and it is therefore inappropriate to regard a tree hazardous merely because decay has been detected. Instead, everyone who carries out hazard assessments needs to be able, as far as possible, to distinguish between those cases of decay that are hazardous and those that are of little or no consequence for safety. In this way, appropriate management decisions can be made, so that hazardous trees are not neglected, and relatively safe ones are not subjected to unnecessary felling or other remedial work.

The fact that not all types of decay are equally devastating as far as tree safety is concerned is borne out by observational knowledge of certain decay fungi. For example, decay in beech trees (*Fagus sylvatica*) caused by *Meripilus giganteus* is frequently associated with root-plate failures, whereas decay caused in pedunculate oak (*Quercus robur*) by *Fistulina hepatica* is associated with failure only in rare, very advanced cases. Our light microscopy studies indicate that various host species can be affected differently by one and the same fungus (Schwarze *et al.* 2004). The clearest example of this is *Inonotus hispidus* which frequently causes failure in the stems or branches of common ash (*Fraxinus excelsior*) and walnut (*Juglans regia*), but which is rarely reported to cause failure in London plane (*Platanus x hispanica*) even in individuals that have been infected over many years. We previously stressed the need to understand the reasons why the mechanical properties of partially decayed wood differ according to the combination of host and fungal species (Schwarze *et al.* 1997). Tree species differ in the properties of their wood anatomy which partly determines the severity and nature of strength loss due to decay. Equally, fungal species differ considerably in a range of attributes, especially in (1) their biochemical systems for degrading components of the woody cell wall, (2) their tolerance of environmental extremes, and (3) their morphology.

A wide range of different types of decay can result from permutations of host species, fungal species and conditions within the wood. Within this spectrum, three main types are commonly recognised: brown rots, white rots, and soft rots. The present review explores parts of the range of variation that each of these encompass, as shown partly by our own use of improved techniques for the preparation and examination of decayed wood by light microscopy (Schwarze *et al.* 2004). The application of these techniques has provided a detailed picture of the decay patterns that occur amongst a wide range of host/fungus combinations (Table 1). The data discussed here come from a selection of twenty four combinations of host and fungal species, all of which are represented

by the accompanying photo-micrographs. These combinations were set up using artificially inoculated wood blocks in axenic conditions. Additionally, some combinations were represented by samples taken from naturally infected material.

For present purposes, those aspects of our studies which have particular relevance to tree hazard assessment will be reviewed in relation to earlier work. In this context our investigations clearly show that, for a better interpretation of the development of decay in trees, studies of individual host-fungus-combinations, involving both host response and fungal invasiveness, should be addressed. The comparative study of challenged reaction and barrier zones in artificially incubated wood and in standing trees appears to provide a means of elucidating the potential mechanisms whereby fungi may overcome host defences, while also determining whether these mechanisms operate in nature.

When trees are injured and infected they chemically strengthen their boundaries that resist spread of infections in wood extant at time of wounding, - reaction zone - and then trees form another new anatomical and chemical boundary that separates the infected wood from the new healthy wood that continues to form - barrier zone. This defence process in trees is called compartmentalization. Based on such observations, the CODIT-model (Compartmentalization of Decay in Trees) was developed and introduced by Shigo and Marx (1977).

Many aspects of the interactions between host wood structure and fungal activity can be revealed by high resolution light microscopy, and this technique has provided much of the information discussed here. Light microscopy permits rapid view of many cells with minimal to moderate specimen preparation. Although this can also be accomplished with scanning electron microscopy (SEM), light microscopy in combination with differential staining techniques allows observation of early stages of selective delignification or features within the cell wall, such as soft-rot cavities. Also, one critical decay feature, the loss of birefringence during brown rot requires polarized light microscopy (Wilcox 1993a, 1993b).

2. Construction of the woody cell wall

A full understanding of the interactions between wood decay fungi and living trees requires anatomical, physiological and biochemical studies. The anatomy of sound wood has been described, by, e.g., Grosser (1977) and Carlquist (1988), and the relationship between wood anatomy and microbial colonization and decomposition has been reviewed elsewhere (Eriksson *et al.* 1990; Daniel 2003).

Gymnospermous wood is relatively homogeneous in structure and consists primarily of tracheids, uniseriate xylem rays, and in some genera, axial parenchyma and epithelial cells surrounding resin canals (Table 2). Tracheids are dual-purpose cells combining properties of both mechanical support and water conduction. By comparison, angiospermous wood is more heterogeneous, and its water conducting functions are served by vessels, while fibres or fibre tracheids mainly supply mechanical strength and support. Parenchyma is a more prominent feature in angiospermous than in

Table 1 – Stains and methods applied in microscopy for detection and interpretation of wood decay types

Stain/method	Information	Comments	Reference
Safranin/picro aniline blue	Staining of hyphae in wood	Woody cell walls appear red, hyphae blue.	Cartwright & Findlay 1929
Wiesner reaction phloroglucinol/HCL Also known as Maule reaction	Qualitative detection of lignin in cell walls	Means of distinguishing between the wood of angiosperms and gymnosperms	Sharma 1922
Safranin (also Safranin O or basic red 2)	Semi-qualitative detection of lignin in cell walls	Safranin is used as a counterstain in some staining protocols, colouring all cell nuclei red. This is the classic counterstain in a Gram stain.	Cartwright & Findlay 1929; Jensen 1962
Safranin-astra blue	Early stages of white rot i.e. selective delignification	Safranin stains lignin regardless of whether cellulose is present, whereas astra blue stains cellulose only in the absence of lignin.	Srebotnik & Messner 1994; Schwarze and Engels 1998
Chlor-zinc-iodine	Distinguishes tension wood fibres from normal fibres	Tension wood fibres stain purplish or reddish brown, normal wood stains yellow.	Chow 1947
Ruthenium red and hydroxylamine-ferric chloride	Determination and distribution of pectin	Hydroxylamine-ferric chloride reaction is enhanced by methylation of sections in a hot solution of absolute methyl alcohol containing 0.5 M HCL.	Jensen 1962; Schwarze & Fink 1998
Toluidine blue	Qualitative determination of lignin and its distribution		Herr 1992; Schwarze et al. 2000a, Schwarze et al. 2000b
Polarized light	Early stages of brown rot	Loss of birefringence due to degradation of cellulose	Lohwag 1937; Schulze & Theden 1937; Wilcox 1978, 1993a; Schwarze et al. 2000a; Schwarze et al. 2003
UV-microscopy	Lignin monomer composition	UV-absorbance spectrum is measured in the wavelength range 250–300 nm.	Scott et al. 1969; Scott & Goring 1970; Schwarze et al. 2000a, Schwarze et al. 2000b
Mäule colour reaction potassium permanganate	Semi-qualitative determination of syringyl lignin monomer units within the cell walls	Absorbance of visible spectra in the wavelength ranges 435–70 nm.	Mäule 1901; Nakano and Meshitsuka, 1978; Iiyama & Pant 1988; Tabake et al. 1992; Wu et al. 1990; Schwarze et al. 2000a, Schwarze et al. 2000b
Autofluorescence	Semi-qualitative determination of lignin	Unstained sections are observed with a fluorescence microscope	Schwarze & Baum 2000; Baum & Schwarze 2002
Secondary fluorescence	Early and advanced stages of delignification	Section are stained with acridine-orange and observed with a fluorescence microscope	von Aufsess 1968

gymnospermous wood, with most genera having multiseriate xylem rays and varying amounts of axial parenchyma (Table 2).

The wood cell wall is organised in layers of different thicknesses and different ratios of cellulose, hemicellulose and the matrix material lignin (Brändström 2001; Harada & Coté 1985; Wardrop 1964; Fig 1). The cell wall proper consists of a thin primary wall, to which a much thicker secondary wall, consisting of three layers (S_1 , S_2 and S_3), is added after initial formation of the cell. The walls of adjacent cells are bonded together by the middle lamella (Figs 1–3), consisting of lignin, calcium and pectic substances (Fig 4). These compounds are amorphous and are therefore non-birefringent, i.e. they do not appear bright when placed in a light beam between polarizing filters (Nicols). The main structural component of the walls of young wood cells is cellulose, a polysaccharide whose long thread-like

molecules are made up from glucose molecules joined end to end by hydroxyl linkages without any side branching. This forms a largely crystalline structure, which has the optical property of birefringence and so appears bright when viewed between crossed Nicols (Fig 2). Within the different cell wall layers, cellulose exists as a system of fibrils of 3–4 nm diameter aggregated in larger structural units. The cellulose microfibrils are helically wound at different angles in the various layers of the cell wall (Fig 3). These different helical windings are thought to contribute to the mechanical resilience of the wood. The degree of polymerization is also mechanically important, since it is highly correlated with tensile strength. The cellulose molecules are surrounded by lignin and hemicelluloses. The major hemicelluloses in coniferous wood are galactoglucomannan, glucomannan and arabinoglucuronoxylan. Other softwood hemicelluloses are arabinogalactan,

Table 2 – Cell content in percent of different tree species (Wagenführ, 1999)

Tree spp.	Vessels	Fibres Tracheids	Xylem ray parenchyma	Axial parenchyma	Density g/cm ³
<i>Abies alba</i>	–	90	10	scanty	0.45
<i>Larix decidua</i>	–	91	8	1	0.59
<i>Picea abies</i>	–	95	4	1	0.47
<i>Pinus sylvestris</i>	–	93	7	–	0.52
<i>Pseudotsuga menziesii</i>	–	93	7	–	0.51
<i>Taxus baccata</i>	–	86	14	–	0.67
<i>Acer pseudoplatanus</i>	7	76	17	scanty	0.63
<i>Aesculus hippocastanum</i>	8	76	15	1	0.55
<i>Alnus glutinosa</i>	29	58	12	1	0.55
<i>Betula pendula</i>	24	64	10	2	0.65
<i>Capinus betulus</i>	10	66	22	2	0.83
<i>Castanea sativa</i>	26	56	17	1	0.63
<i>Fagus sylvatica</i>	31	37	27	5	0.72
<i>Fraxinus excelsior</i>	12	62	15	11	0.69
<i>Juglans regia</i>	12	64	16	8	0.68
<i>Liquidambar styraciflua</i>	55	26	18	1	0.52
<i>Platanus x hispanica</i>	29	31	31	9	0.62
<i>Populus nigra</i>	34	56	10	scanty	0.45
<i>Pyrus communis</i>	27	49	16	8	0.74
<i>Quercus robur</i>	8	58	29	5	0.69
<i>Robinia pseudoacacia</i>	15	58	6	21	0.77
<i>Salix alba</i>	52	31	17	scanty	0.35
<i>Sorbus aucuparia</i>	32	47	20	1	0.75
<i>Tilia platyphyllos</i>	17	72	9	2	0.53
<i>Ulmus glabra</i>	29	51	14	6	0.68
<i>Ochroma pyramidale</i>	4	4	18	74	0.20

xyloglucan and other glucans. Other polysaccharides are pectins, composed mainly of linearly connected β -1,4-D-galacturonase acid units and their methyl esters, interrupted in places by 1,2-linked L-rhamnose units (Green *et al.* 1996). In wood cells, a major part of the pectic substances occurs as polygalacturonic acid in the middle lamella usually together with Ca²⁺ ions in the form of calcium pectate (Fengel & Wegener 1989).

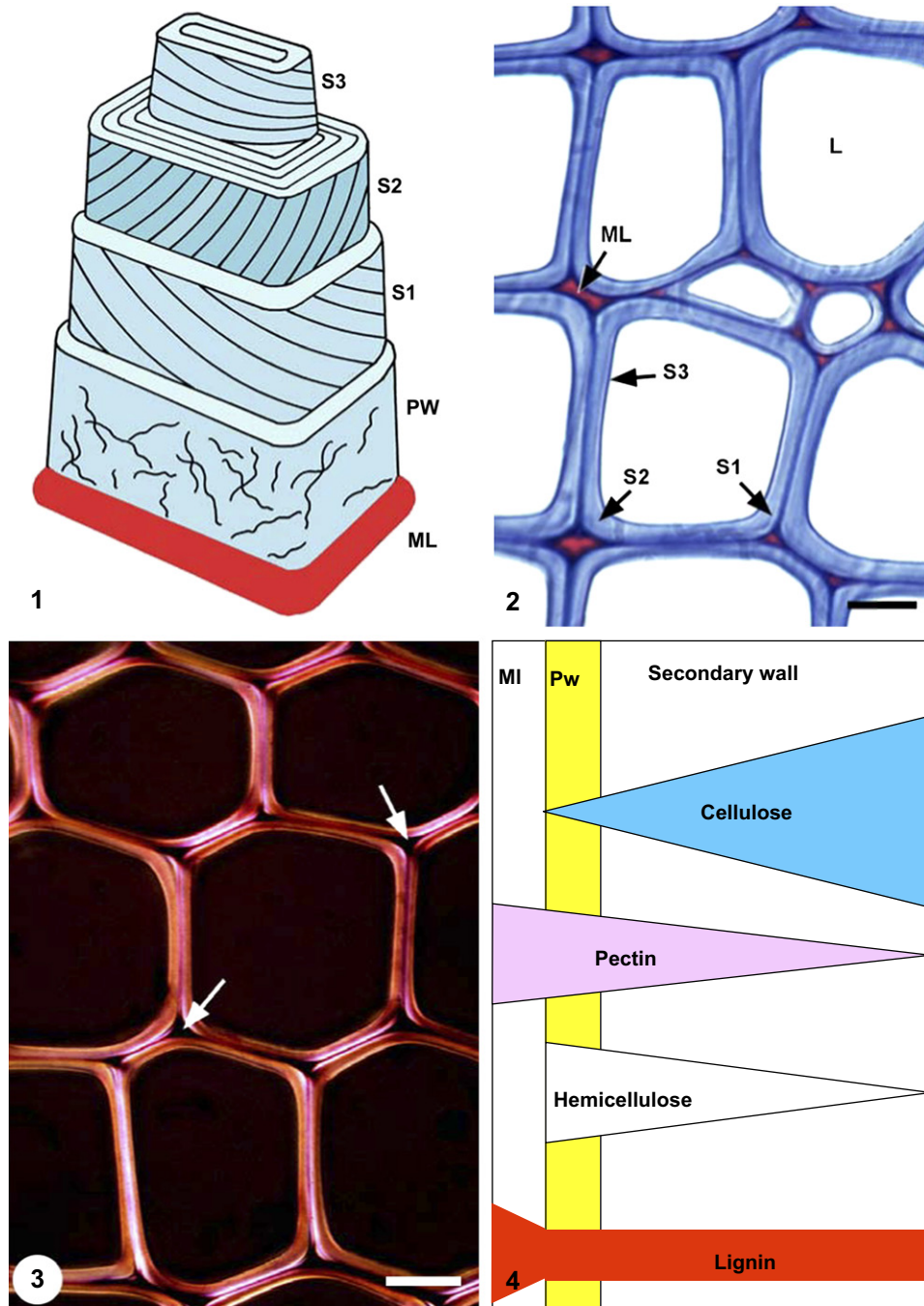
The arrangement and interactions of these chemical constituents at the nanometer level have not been completely resolved. Images presented by e.g. Fahlén and Salmén (2002) or Ruel and Goring (1978) showed that the thickest cell wall layer (S₂) solely consists of concentric lamellae, whereas investigations by Larsen *et al.* (1995), Sell and Zimmermann (1993) and Schwarze and Engels (1998) with the aid of wood decay fungi revealed a radial arrangement of the fibril/matrix structure (perpendicular to the compound middle lamella). In contrast to both models, a random texture of cell wall components within the S₂ layer of softwood tracheids was recently described by Donaldson and Frankland (2004). It has been suggested that different organisation patterns coexist (Sell & Zimmerman 1998; Brändström 2001; Singh & Daniel 2001; Zimmermann *et al.* 2006).

Wood is an exceptionally difficult resource to decompose principally because it contains very low concentrations of nitrogen (C:N of birch is 55 and sycamore 401). Nitrogen is the limiting factor for fungal growth being required for enzyme production. Predation of nematodes and other microfauna organisms add extra protein (nitrogen) to the system (Hutchison & Barron 1997; Barron 1992, 2003). In addition, wood contains potentially fungitoxic compounds, which are deposited in the heartwood or within reaction or barrier zones at the

host-fungus-interface. In angiospermous trees, the toxic compounds are usually polyphenols or tannins. Gymnospermous wood contain a range of phenolic compounds such as terpenes, stilbenes, flavonoids and tropolones. The most toxic of the tropolones are the thujaplicins that act as uncouplers of oxidative phosphorylation. Decay fungi can degrade these compounds, but fungal species differ in their abilities. Also, different components may be variously active or inactive under a given set of environmental conditions. In this context, the availability of oxygen, which may be greater in some parts of the wood than others, is particularly important (Boddy & Rayner 1983).

Lignin composition in different tree species and its effect on wood decay resistance

During the maturation of woody cells, all the layers of the wall together with the middle lamella are, to a greater or lesser extent, impregnated with lignin. Lignification is especially pronounced in the compound middle lamella, where it can exceed 80 % in localised regions e.g. where it forms an infilling between the rounded corners of the fibres (Fergus & Goring 1970a, b). Taking the cell wall as a whole, the typical composition is about 50 % cellulose (of the dry weight of wood), 25 % lignin, 20–25 % hemicellulose, 1–4 % pectin (Fig 4). Generally the lignin content of gymnospermous wood is higher than that of angiospermous wood. Transmission electron microscopy (TEM) has shown clear differences between angiosperms and gymnospermous woods in terms of micro-distribution of the middle lamella lignin, hardwoods showing much greater



Figs. 1–4 – I. Woody cell wall. (1) Conventional cell-wall model which distinguishes five cell-wall layers. These are the middle lamella (ML), the primary wall (PW), and the three-layer secondary wall (S): outer (S_1), middle (S_2) and inner secondary wall layer (S_3). (2) Transverse section (T.S.) of earlywood tracheids. ML, middle lamella; S_1 , S_2 , S_3 , outer, middle and inner layers of secondary wall. L, lumen. Bar, 10 μm . (3) T.S. of earlywood viewed between crossed Nicols. Note secondary walls appear bright, whereas the amorphous compounds in the middle lamella are non-birefringent (arrows). Bar, 10 μm . (4) Diagrammatic representation of the relative distribution of the main cell wall constituents within the different layers of the cell wall.

heterogeneity than conifers (Daniel *et al.* 1991; Singh and Schmitt 2000).

The lignin component presents the most significant barrier to wood decay as it is a complex polymer that encrusts the cell walls, preventing access of low molecular weight diffusible

agents, which are required for decomposition of cellulose and hemicelluloses. Lignins are a heterogeneous class of compounds (Fengel & Wegener 1989) and the nature of the lignin has a great influence on wood decomposition by fungi. Lignins are polymers of phenylpropane units: guaiacyl (G) units from

the precursor trans-coniferyl-alcohol, syringyl (S) units from trans-sinapyl-alcohol, and p-hydroxyphenyl (H) units from the precursor trans-p-coumaryl alcohol. The exact composition of lignin varies widely with species. In addition to classification as softwood, hardwood and grass lignins, lignins can be divided into two major groups: guaiacyl lignins and guaiacyl-syringyl lignins (Gibbs 1958). Guaiacyl lignins are predominantly polymerization products of coniferyl alcohol while guaiacyl-syringyl lignins are composed of varying parts of aromatic nuclei guaiacyl and syringyl, together with small amounts of p-hydroxyphenyl units (Fengel & Wegener 1989). The ratios of these monomers vary between individual cell types and cell wall layers and strongly influence the resistance to decay fungi (Fukazawa 1992).

Gymnospermous wood consists almost exclusively of guaiacyl monomers, whereas angiospermous wood consists of approximately equal ratios of guaiacyl and syringyl monomers (Whetten & Sederoff 1995). For Norway spruce (*Picea abies*) a ratio G:S:H = 94:1:5 has been reported (Erickson et al. 1973). The monomeric composition of lignin in different tree species, revealed by UV microscopy (Baum 2001) relates to different evolutionary stages of the water conducting system of the xylem (Schwarze 2001). According to Braun (1970) the xylem of evolutionarily primitive xylem consists of tracheids that perform a dual function, i.e. conduct water and provide strength and support, whereas trees with a more advanced xylem possess cell types that are highly specialised. Thus, the first evolutionary stage of the water conducting system is represented by Norway spruce that consists almost entirely of tracheids, which have an unspecialised function providing both water conduction, strength and support (Fig 5). Typically, for conifers, e.g. *Abies alba* (silver fir), *Larix decidua* (European larch), Norway spruce, *Pinus sylvestris* (Scots pine) and *Pseudotsuga menziesii* (Douglas fir), *Taxus baccata* (yew) guaiacyl lignin dominates within cell walls with highest concentrations in the latewood tracheids (Baum 2001; Fig 5).

The xylem of beech (*Fagus sylvatica*), birch (*Betula pendula*), large-leaved lime (*Tilia platyphyllos*), Sweetgum (*Liquidambar styraciflua*), London plane, sweet chestnut (*Castanea sativa*) and walnut (*Juglans regia*) represents the second evolutionary stage of the water conducting system (Fig 6). The xylem consists of water conducting vessels embedded in a matrix of fibre tracheids that provide strength, support and water conduction. Although both cell types conduct water, only vessels have higher concentrations of guaiacyl lignin, whereas fibre tracheids and parenchyma cells have low-moderate concentrations of syringyl lignin (Baum 2001). The homogeneous distribution of syringyl lignin throughout the cell walls may explain why a great range of wood decay fungi can colonize and readily degrade beech wood.

The xylem of pedunculate oak represents the third evolutionary stage (Fig 7), consisting of cell types with specialised functions. The xylem of oak is ring-porous and contains radial bands of tissue consisting of earlywood and latewood vessels within a matrix of thin-walled fibre tracheids, which combine properties of water conduction and, to a lesser degree, strength and support. Libriform wood fibres are located between the vessel-fibre tracheid regions and provide most strength and support. Examination of the lignin monomer composition of control sections, using UV-microscopy,

showed that fibre tracheids had a higher guaiacyl monomer content, whereas libriform wood fibres had markedly higher syringyl monomer content (Schwarze et al. 2000a; Baum 2001).

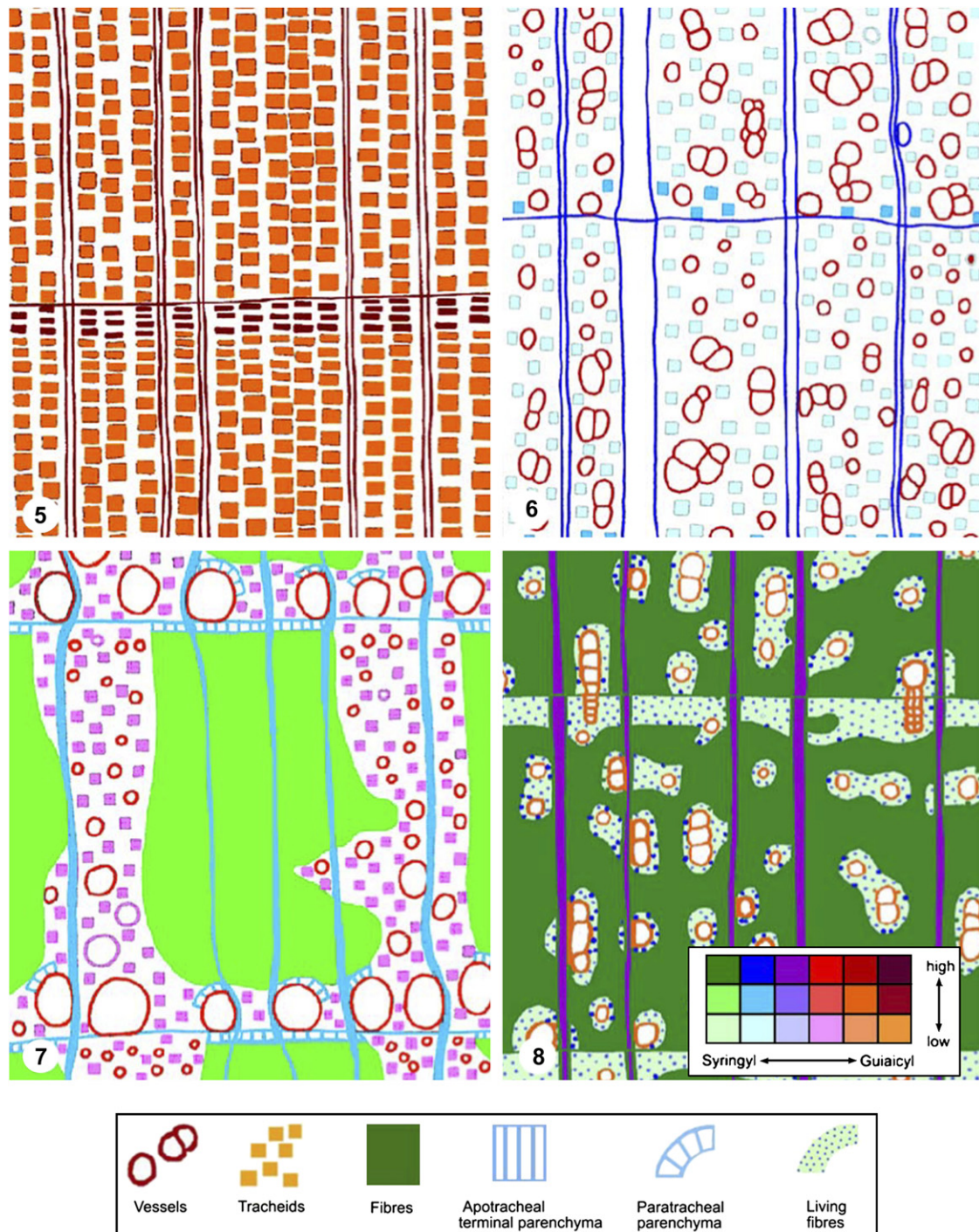
The xylem of sycamore (*Acer pseudoplatanus*), horse chestnut (*Aesculus hippocastanum*), ash and balsa (*Ochroma pyramidale*) represent the most advanced evolutionary stage (Fig 8). All cells have a highly specialised function and either conduct water or provide strength and support. Thus, vessels exclusively conduct water and are isolated by a sheath of living libriform wood fibres and embedded within a matrix of dead libriform wood fibres that provide strength and support. Typically, syringyl lignin dominates (Schwarze et al. 2000b; Baum 2001).

Knowledge of the lignin composition of different cell types of wood allows interpretation of degradation modes and allows prediction of whether the xylem of a specific host is resistant or susceptible to a certain decay type (Obst et al. 1994). For example, the vessels and the compound middle lamella in the cell walls have a very high concentration of guaiacyl units, and thus are particularly resistant to fungi that cause soft rot and other decay types (Liese 1961; Faix et al. 1985; Blanchette et al. 1988; Nilsson et al. 1989; Schwarze et al. 1995b), whereas wood that consists predominantly of syringyl-rich libriform wood fibres is highly susceptible to xylariaceous soft-rot fungi (Schwarze et al. 2004). Weight losses of sapwood blocks excised from Norway spruce, beech, oak and Norway maple and incubated with three xylariaceous fungi *Daldinia concentrica*, *Kretzschmaria deusta* and *Xylaria longipes* varied significantly depending on the lignin composition (Fig 9). In wood with a higher ratio of syringyl lignin, e.g. beech and Norway maple, weight losses were significantly higher than in the guaiacyl richer wood of Norway spruce and oak (Fig 9). These results are in good agreement with studies on tropical xylariaceous taxa in the genera *Biscogniauxia*, *Hypoxylon*, and *Xylaria* that were evaluated for their ability to produce wood-decay enzymes and effect mass loss and lignin solubilisation in angiospermous and gymnospermous wood (Pointing et al. 2003). All xylariaceous taxa were capable of cellulose and xylan hydrolysis, but few produced enzymes involved in lignin breakdown. The xylariaceae failed to cause mass loss in gymnosperm wood, but several did so in angiospermous wood during a six month *in vitro* exposure, though mass losses were about 20 % of those caused by basidiomycetes (Pointing et al. 2003).

As the sapwood of most tree species has low amounts of extractives it is generally considered susceptible to decay (European standard EN 350-1 1994). However, clearly durability of sapwood can vary greatly between different wood species. Moreover, recent studies show, that certain cell types, e.g. parenchyma cells, which have been regarded as strongly susceptible to decay, may be highly resistant to brown rot fungi. Such differences have not been considered in the past when classifying wood durability. Evidence will be provided here showing that even slight variations in the lignin composition within cell types and/or cell wall layers, and the proportion of parenchyma cells may influence the durability of wood.

3. Wood decay types

Fungal decay types fall into three categories according to their mode of degradation of the woody cell walls: brown rot, white



Figs. 5–8 – II. Lignin composition. Evolutionary stages in the development of hydraulic systems in trees (Braun, 1970). (5). Stage 1 tracheids without vessels e.g. Norway spruce. (6) Stage 2 tracheids with vessels e.g. beech. (7) Stage 3 tracheids, vessels and fibres e.g. oak. (8) Stage 4 fibres e.g. sycamore. Note: Many authors use the term fibre-tracheids to describe the cell type here by Braun as tracheids in deciduous trees. Colour spectrum represents the monomeric composition of lignin within the wood cells of each evolutionary stage.

rot and soft rot. White rot is subdivided into simultaneous rot and selective delignification, and soft rot is divided into types 1 and 2 (Figs 10–13). The ability to oxidize phenolic compounds extracellularly was traditionally used to differentiate white rot fungi from brown rot species. For this purpose a rapid

screening test for white rot fungi based on polyphenol activity, the Bavendamm test has been used (Bavendamm 1928). Today there are great ranges of reagents that are used to identify phenoloxidases of wood decay fungi in pure culture (Etheridge 1957; Nobles 1958; Käärnik 1965; Stalpers 1978; Rayner and

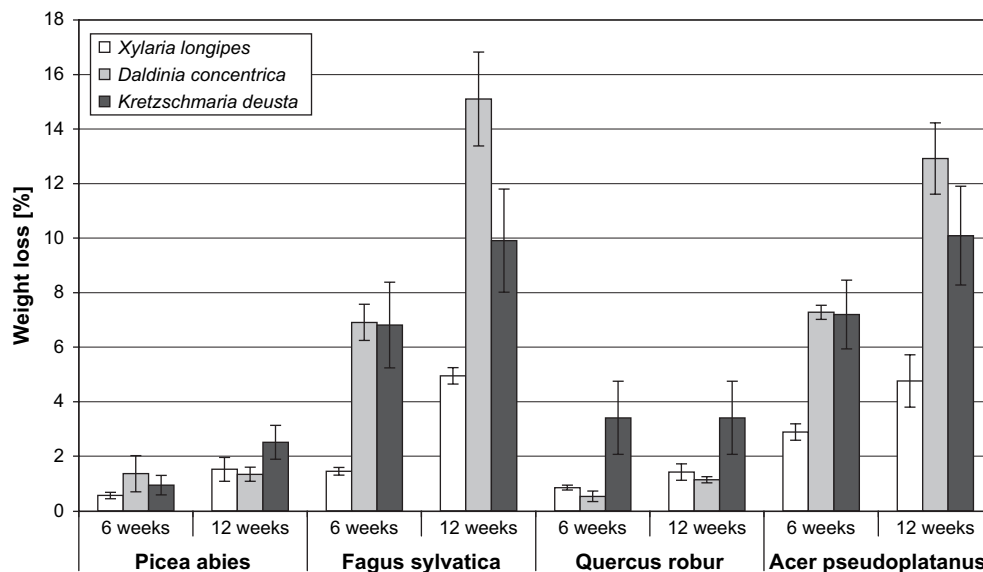


Fig. 9 – Lignin composition. Weight losses of sapwood wood blocks of Norway spruce, beech, oak and sycamore artificially incubated with three xylariaceous wood decay fungi.

Boddy 1998). The following sections present a summary of the histological research on wood decay and describe key findings.

4. Colonization of wood by hyphae

The axial alignment of tracheids, vessels and fibres and the radial arrangement of the xylem ray parenchyma facilitate access into the wood and allow widespread distribution of hyphae within the xylem (Rayner & Boddy 1988; Schwarze et al. 2004). Access to adjacent cells occurs via pit apertures, or direct penetration may take place directly through the cell wall. In wood of Norway spruce (*Picea abies*), abundant longitudinal hyphal growth of *Stereum sanguinolentum* occurred rapidly within the broad cell lumina of the earlywood tracheids and radially within the xylem ray parenchyma (Fig 14; Kleist & Seehaan 1997; Schwarze 2004). By contrast, hyphal growth in the cell lumen of thick-walled latewood tracheids was sparse as the narrow cell lumina and limited numbers of bordered pits passively hamper colonization (Fig 14). In angiospermous wood, white rot fungi showed a tendency to colonize cells via apertures of simple pits or bordered pits which are subsequently enlarged (Fig 15) and mistaken for bore holes. *Fomes fomentarius* induces abundant boreholes in colonized wood of beech and pedunculate oak. Formation of boreholes by specialised cell wall degrading hyphae has been described in the literature (Liese 1970; Schwarze et al. 2004). These were initiated by fine penetration hyphae, less than 0.5 µm diameter, which penetrated the cell wall by means of lignolytic enzymes, which were released at the hyphal tip (Schwarze et al. 2004). Subsequently, bore holes progressively enlarged by the secretion of enzymes from the general surface of the hypha. At a more advanced stage of decay, cracks often developed between adjacent boreholes in the radial cell walls, and the boreholes eventually coalesce. *Fomes*

fomentarius can be classified as a weakly invasive decay fungus and is found predominantly on weakened hosts that are over-mature or declining. In the presence of polyphenols within reaction zones of healthy trees, growth of the fungus is hampered and boreholes are not formed (Fig 46) in part explaining why it is only weakly invasive.

Not all decay fungi have the enzymatic capacity to form boreholes in wood cells. Some members of the Xylariaceae, such as *Kretzschmaria deusta*, are unable to pass laterally between the cell walls of various host species, except via pits (Wilkins 1936; 1943; Schwarze et al. 1995b; Schwarze & Baum 2000; Baum & Schwarze 2002). Vessels and the compound middle lamella have a very high concentration of guaiacyl, and thus are particularly resistant to soft rot fungi such as *K. deusta* (Blanchette et al. 1988; Nilsson et al. 1989; Schwarze et al. 1995b). Its inability to form boreholes through the compound middle lamella (Fig 16) seems consistent with its tendency to leave this layer as an intact skeleton even at quite an advanced stage of decay (Nilsson et al. 1989; Schwarze et al. 1995b). Detection of decay by *K. deusta* during tree hazard assessment is difficult (Fig 17). Due to the inconspicuous ascocarps and brittle nature of the decayed wood infected trees do not show typical defect symptoms, e.g. bulges or bottle butt. Moreover, detection of decay with acoustic diagnostic devices is also difficult as it causes little change in sound transit times probably because the mechanical properties modulus of elasticity and gross density, are altered proportionally to each other (Schwarze and Fink 1994; Schwarze et al. 1995b).

Water saturation of wood impedes the availability of oxygen necessary for colonization and wood decay (Boddy & Rayner 1983; Boddy 1992). Storage of logs under water sprinkling is therefore used as a wood protection method in forestry (Moltensén 1971; Peek & Liese 1974; Webber & Gibbs 1996; Groß & Metzler 1995). However, *Armillaria* spp. can cause sapwood decay in wood stored under water sprinkling (Metzler 1994). Light microscopy revealed that decay of water-saturated wood by

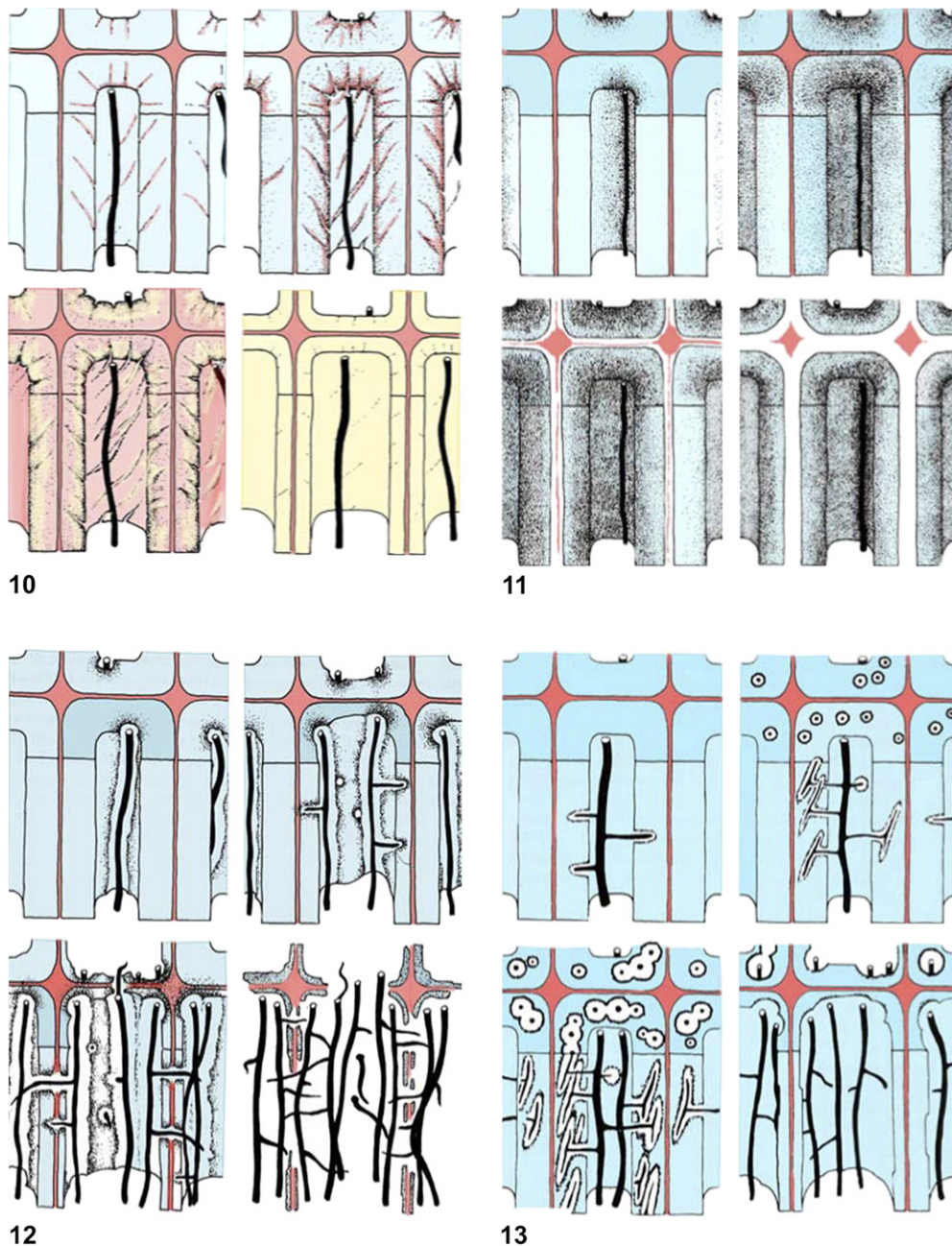


Fig. 10–13 – III. Schematic drawings showing micro-morphological features of different decay types. (10) Brown rot by *Fomitopsis pinicola*. At an early stage of decay low molecular weight substances are secreted by hyphae growing on the S_3 layer and diffuse radially into the cell wall. At a more advanced stage enzymes have penetrated into the entire secondary wall, this involving extensive breakdown of hemicellulose and cellulose. Wood shrinkage leads to the formation of numerous cracks and clefts within the secondary wall. Even at advanced stages the S_3 remains intact and a matrix of modified lignin persists. (11) Selective delignification by *Heterobasidion annosum*. At an early stage of decay low molecular weight substances (shown as dots) diffuse into the secondary wall from hyphae growing in the lumen. These initiate the degradation of hemicellulose and lignin within the secondary wall, also extending to the middle lamella. At advanced stages the preferential degradation of pectin and lignin results in the separation of individual cells from one another. Initially cellulose remains intact. (12) Simultaneous rot by *Fomes fomentarius*. At an early stage degradation occurs in the immediate vicinity of abundant hyphae growing within the lumen. The cell wall is progressively degraded from the lumen outwards. Individual hyphae penetrate into the cell wall at right angles to the cell axis. The cell wall becomes increasingly thinner, and numerous boreholes appear between adjacent cells. At a more advanced stage degradation is hampered by the strongly lignified compound middle lamella. (13) Soft rot by *Kretzschmaria deusta*. At an early stage of decay hyphae diffuse into the secondary wall. Branching and orientation of hyphal growth parallel to the orientation of the cellulose microfibrils in the S_2 layer. Degradation of the cell wall around hyphae leads to the formation of cavities with conically shaped ends. At an advanced stage of decay the secondary wall is nearly completely broken down, whereas the guaiacyl-rich compound middle lamella persists. (10–13) reproduced from Schwarze et al. (2004) by permission of the Rombach Verlag.

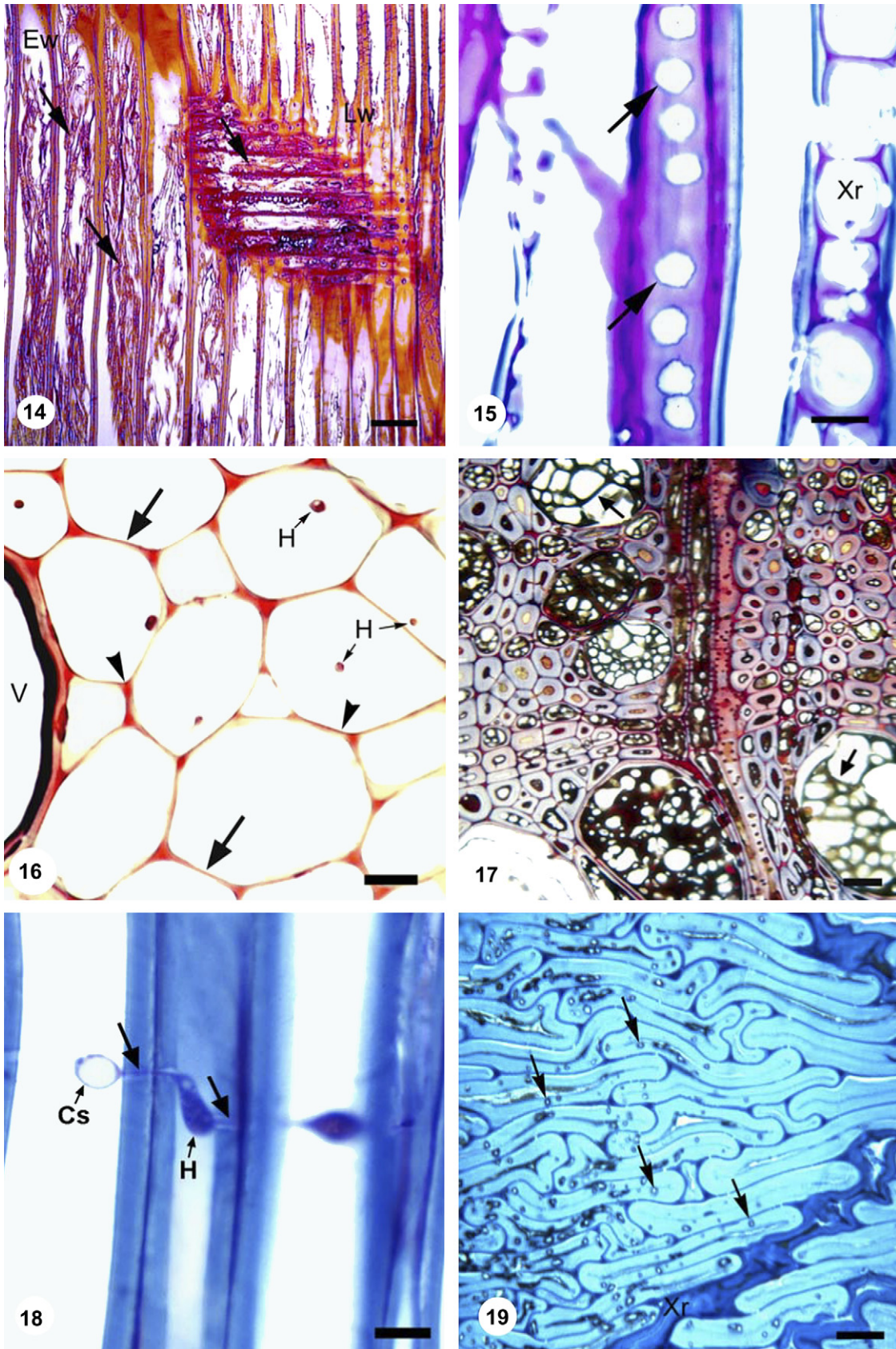


Fig. 14-19 – IVa. Colonization. (14) Radial longitudinal section (R.L.S.) showing hyphal colonization of wood of Norway spruce by *Stereum sanguinolentum*. Note: abundant hyphal growth in the cell lumen of earlywood tracheids (Ew) and xylem ray parenchyma (arrows), whereas latewood tracheids (Lw) are only weakly colonized. Bar, 10 μm. (15) T.L.S. showing formation

Armillaria spp. was associated with the formation of radial tubular air canals extending from the cambial region into the sapwood (Metzler & Hecht 2004). Their light colour results from different refraction of light in gas-filled versus water-filled wood structures. These structures were formed around wood rays by a tubular sheath of pseudoparenchymatous mycelium (Metzler & Hecht 2004).

Formation of chlamydo spores may be crucial to survival under dry or very moist conditions for a range of wood decay fungi. Both *Oligoporus placenta* (= *Poria placenta*) and *Antrrodia carbonica* produce chlamydo spores, which facilitate their survival during prolonged exposure to elevated temperatures and other adverse conditions (Powell 2002). The brown rot fungus *Laetiporus sulphureus*, formed chlamydo spores in heartwood of robinia (*Robinia pseudoacacia*) (Fig 18). During early stages of colonization, most hyphae grew within the lumen of the libriform wood fibres. Individual hyphae grew transversely via minute bore holes to adjacent cells and subsequently produced single chlamydo spores in the cell lumen (Schwarze et al. 2004). These thick-walled resting spores allow survival of the fungus in time rather than space.

Wood colonization and its relevance for developing environmentally friendly methods for wood protection

The design of environmentally benign methods for preserving wood in service requires an understanding of the precise sequence of the biochemical events that occur as wood is colonized by decay fungi (Zabel & Morrell 1992; Green et al. 1997). Hyphal colonization of wood by decay fungi can be simply counteracted by modifying the wood structure with compression and heat without the need to apply wood preservatives. Thermo-Hygro-Mechanically (THM)-densified wood is a unique engineered wood product (Naviz & Giradet 2000). The resistance of Norway spruce wood to colonization and decomposition by three brown rot fungi, *Coniophora puteana*, *Gloeophyllum trabeum* and *Poria placenta*, was increased by compression of wood specimens in a multi-parameter reactor under saturated steam conditions and post treatment with heat (Schwarze & Spycher 2005). In comparison to controls, wood weight losses induced by *Coniophora puteana*, *Gloeophyllum trabeum* and *Poria placenta* were significantly lower in compressed wood, post-treated at 180 °C. Microscopical examination of colonized THM densified wood showed that the differences could be partly attributed to the restriction of fungal growth by the occlusion of tracheid lumina. Complete occlusion of all cell lumina is needed to prevent brown-rot activity, since degradative substances can diffuse into the cell wall from a single hypha in a cell lumen. However,

if a high proportion of lumina are completely occluded, this will clearly restrict fungal colonization overall, sufficiently to explain the reduction in decay rate. Complete cell occlusion does not affect decay rates in THM-densified wood exposed to soft rot Type 1, as hyphae typically grow within the secondary walls of colonized wood. In THM-densified wood of Norway spruce and beech occlusion of the cell lumina was simply counteracted by directional growth of hyphae within the cell wall (Fig 19). Soft rot commenced from the outer wood surfaces and cavity formation was not found in deeper regions of the wood samples.

Hydrolysis of wood pectin from the tori of pit membranes has been proposed as an essential step in the colonization of wood by brown rot fungi (Daniel 1994; Green & Highley 1997). Pit membranes in the sapwood of wood cell walls represent a readily available source of non-lignified carbohydrates, i.e. pectin and cellulose. Studies on pectin-hydrolyzing enzymes in wood decay fungi are rare, probably because of the relatively low content (<4%) of pectin in the solid wood (Shanley et al. 1993). The primary location of calcium is associated with pectin, which is found in pit tori, the middle lamella, cell corners, xylem ray parenchyma, and resin canal parenchyma. (Militz 1993a, b; Bailey & Reeve 1994; Daniel et al. 1996; Schwarze & Fink 1998). The low pectin content may be misleading in terms of its significance during early stages of decay. Previous studies have demonstrated that most brown- and white rot fungi have the capacity to hydrolyze the pectin in pit membranes during incipient decay, which facilitates colonization (Cowling 1961; Wilcox 1978; Green & Clausen 1999; Schwarze & Landmesser 2000; Schwarze & Fink 1998). Thus, Cowling (1961) and Wilcox (1978) reported that during early stages of brown rot by *Postia* sp., the hyphae ramified the entire wood block prior to 5% weight loss, largely by penetration of simple and bordered pits.

Pectin is a good chelator of Ca^{++} and acts as a selective binder for Ca^{++} ions in non-lignified tissue. One key to pectin hydrolysis by plant pathogens has been shown to be fungal production of oxalic acid, which lowers the pH of the substrate and chelates calcium ions. Production of oxalic acid may serve a similar role during incipient wood decay as calcium oxalate has been found, by light- and scanning electron microscopy, during both brown rot and white rot decay (Fig 22; Green et al. 1996; Green & Clausen 1999; Schwarze et al. 2006). Commercial pectinases (Pectinol) and *Trichoderma* spp. degrade pectin by the synergistic action of oxalic acid and polygalacturonase. The oxalic acid solubilises the pectin by chelating the Ca^{++} and the polygalacturonase hydrolyses the β -1,4 linkages. Production of polygalacturonase and hydrolysis of bordered pit membranes during incipient decay has been

of boreholes and enlargement of bordered pits (arrows) in fibre-tracheids of beech by *Fomes fomentarius*. Xr, xylem ray. Bar, 50 μ m. (16) T.S. of beech wood naturally infected with *Kretzschmaria deusta* at an advanced stage of decay. A stiff and brittle lignin skeleton consisting of the compound middle lamellae (pointers) and the vessel (v) cell walls persists within the heavily degraded wood. H, hyphae. Bar, 10 μ m. (17) T.S. of beech wood showing pseudosclerotial plates by *Kretzschmaria deusta*. Note hyphal protrusions in cells (arrows). Bar, 50 μ m. (18) R.L.S. showing hyphal growth (arrows) and chlamydo spore (Cs) formation in libriform wood fibres of robinia by *Laetiporus sulphureus*. Note diameter of hypha (H) is greatly reduced during cell wall penetration. Bar, 10 μ m. Figs. Xx-xx Reproduced from Schwarze et al. (1999) by permission of the Rombach Verlag. (19) T.S. showing compressed Norway spruce wood post-treated at 180 °C and incubated with soft-rot fungi. Although most cell lumina are completely occluded by the treatment, hyphal growth and cavity formation (arrows) is apparent within the secondary wall of tracheids. Bar, 20 μ m. Reproduced from Schwarze et al. (2006) by permission of de Gruyter.

demonstrated for a range of wood decay fungi (Green *et al.* 1991, 1995a,b, 1999). Pre-treatment of wood blocks with the selective water-soluble calcium-precipitating agent N,N-naphthaloylhydroxylamine (NHA) or the pectin dye ruthenium red inhibited decay by brown and white rot fungi (Green *et al.* 1997). Targeting inhibition of pit hydrolysis by decay fungi with agents such as NHA or ruthenium red appears to limit fungal access to non-lignified carbohydrates and prevent colonization.

Some white rot fungi have an extraordinary capacity to hydrolyze the pectin in the middle lamella of xylem during incipient stages of selective delignification. Sections stained with ruthenium red and hydroxylamine-ferric chloride revealed that *Meriplius giganteus* preferentially degraded pectin-rich regions of the middle lamellae in xylem ray cells of beech (Schwarze & Fink 1998). In wood of large-leaved lime, such regions were uniformly located in the middle lamellae of axial and ray parenchyma. In beech wood, degradation of pectin-rich middle lamellae commenced after the delignification of secondary walls and resulted in a conspicuous hollowing of multiseriate xylem rays (Schwarze & Fink 1998).

The effects of treating conifer wood with commercial pectinases or bacteria to improve penetration of preservatives have been studied in detail (Bauch *et al.* 1970; Nicholas & Thomas 1968; Johnson 1979; Sharma & Kumar 1979). Commercial pectinase treatment improved preservation penetration of sapwood of Douglas fir by opening pit apertures, as long as treatment was combined with either low pH or a calcium chelator, such as ammonium oxalate or sodium hexametaphosphate (Tschernitz 1973). The most effective tested on finely ground wood of Norway spruce are hydrolases with a broad spectrum of cellulolytic and hemicellulolytic activity (Militz 1993a,b). The application of these enzymes, however, failed to enhance the permeability of solid wood to any useful extent, due to the slowness of their diffusion into wood and to the effect of extractives, adhering to aspirated pits, making them resistant to decomposition (Militz 1993a,b).

Timbers in cooling towers are mainly colonized and degraded by soft rot causing Ascomycota. The basidiomycete *Physisporinus vitreus* decomposed water-saturated timber in the form of a fibrous white-pocket rot in cooling towers (Acker van & Stevens 1996; Schmidt *et al.* 1996, 1997; Schmidt 2006). In the laboratory, the fungus revealed a remarkable colonization pattern. In crosswise piled water-saturated pine wood the fungus decomposed only those parts not surrounded by air (Schmidt *et al.* 1996, 1997). Some isolates of this fungus have an extraordinary capacity to induce significant permeability changes in heartwood of Norway spruce and silver fir after hydrolysis of bordered pit membranes without causing significant wood strength losses (Schwarze *et al.* 2006). Even after six weeks' incubation, when the mass losses induced by both *P. vitreus*-isolates were slight (>1%), the wood permeability had increased, to approximately 300–400 kg m⁻³ in Norway spruce and to 400–680 kg m⁻³ in silver fir respectively (Schwarze *et al.* 2006). Conspicuous qualitative changes in permeability were also apparent from the uptake of the bluish dye Neolan Glaucin E-A (Fig 20). Uptake of the dye within test blocks of silver fir incubated with *P. vitreus* was visually homogeneous but less so in Norway spruce (Schwarze *et al.* 2006; Fig 20). FE-REM studies revealed that uptake of Neolan

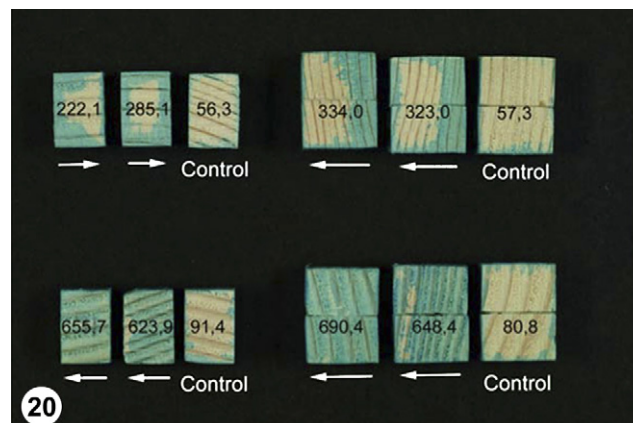


Fig. 20 – IVb. Colonization. Heartwood specimens of *Picea abies* (top) and *Abies alba* (bottom) impregnated with the bluish dye Neolan Glaucin E-A after six weeks incubation with *Physisporinus vitreus*. Numbers refer to radial and tangential uptake of water in kg m⁻³; arrows to the direction of hyphal colonization.

Glaucin E-A was attributable to preferential degradation of pit membranes (Figs 21–24). Complete or partial hydrolysis of bordered pits and crossfield pits in regions of the wood that were stained with Neolan Glaucin E-A was apparent, whereas in unstained regions pit membranes were intact (Schwarze *et al.* 2006). Hyphae entered the pit chamber via the apertures and membranes were subsequently degraded (Figs 22–24). Degradation commenced from the thickened central part of the membrane (torus). Calcium oxalate crystals were regularly observed on hyphae (Fig 22). In silver fir wood they often accumulated within bordered pits in close proximity to hyphae. The preferential degradation of the lignified pit membranes in heartwood by *P. vitreus* is an interesting aspect that could also have industrial benefits for wood protection processes (Rosner *et al.* 1998; Schwarze *et al.* 2006). Further studies are currently in progress with the objective of optimizing the uniformity of wood colonization and duration of incubation, to improve the treatability of conifer wood for preservation, fire protection, UV-protection and dimensional stability or hardness.

5. Brown rot

In brown rot cellulose and hemicelluloses are broken down in the wood substrate, but decomposition of lignin is limited (Rayner & Boddy 1988; Eriksson *et al.* 1990; Green & Highley 1997). Compared with white rot fungi, where commercial application is of greater interest, little is known about the lignin decomposition capacity of brown rot fungi, except for a few reports of the presence of ligninolytic enzymes in brown rot fungi (Kirk 1975; Goñi *et al.* 1993; Schwarze *et al.* 2000a). Brown rot is characterized by rapid degradation of the S₂ layer, but the S₃ layer and the lignin-rich middle lamellae appear to resist degradation (Fig 22). (Meier 1955; Liese 1970; Highley *et al.* 1985; Eriksson *et al.* 1990; Schwarze *et al.* 2004). Because of the preferential decomposition of carbohydrates, the decayed wood acquires a brittle consistency, cracks into cubes and

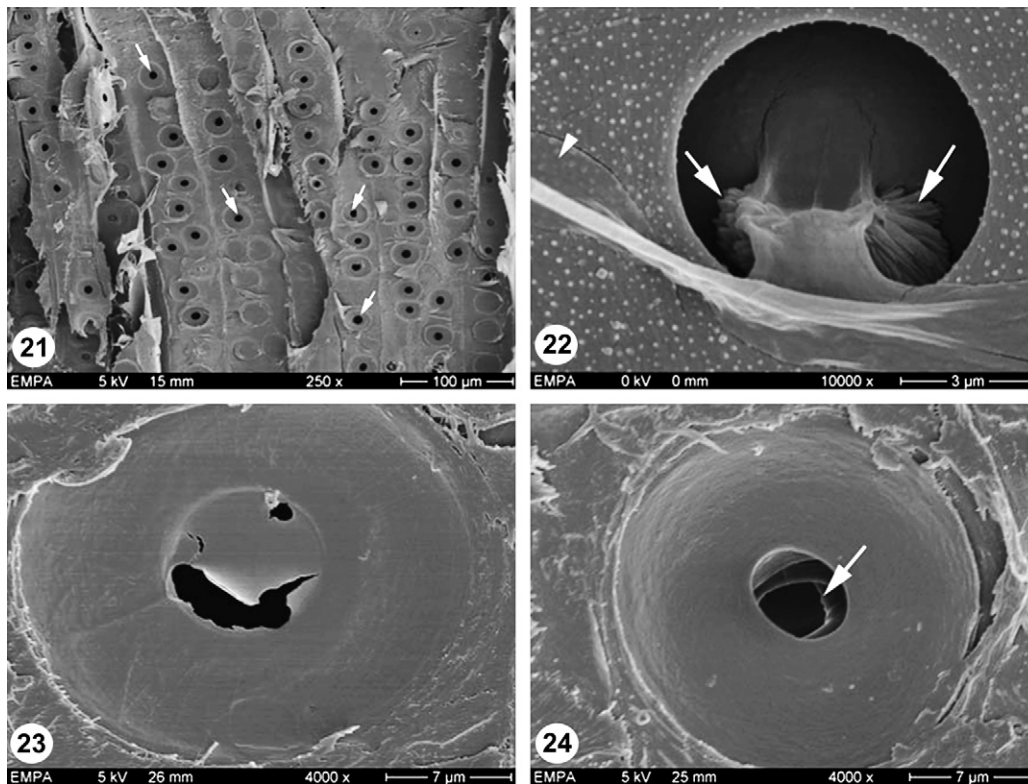


Fig. 21–24 – Colonization. Scanning electron micrographs (5kV) showing bordered pits in *Picea abies* and *Abies alba* after six weeks incubation with *Physisporinus vitreus*. (21) Note degraded tori (arrows) within bordered pits. (22) Hyphae entered the bordered pits via the aperture and degraded the torus; lysis of the warty layer and calcium oxalate crystals in close proximity to hyphae within bordered pit chambers (pointers). (23–24) After twelve weeks, most bordered pits showed partial to complete dissolution of pit membranes, so that both apertures of pit of pit pairs were occasionally exposed (arrow). Figs 20–24 reproduced from Schwarze et al. (2006) by permission of de Gruyter.

finally crumbles into powder. The modified lignin remaining gives the decayed wood its characteristic colour and consistency. Interestingly, only 6 % of all described wood decay fungi are known to cause a brown rot, and most belong to the Polyporaceae (Gilbertson 1980; Rayner & Boddy 1988). Moreover, they are predominantly associated with gymnosperms, whereas white rot fungi are associated with angiosperm trees (Gilbertson 1980; Watling 1982).

The correlation of brown rots with conifers coincides with the relatively small number of species concerned and with the predominantly northern distribution of these fungi compared with the tropical distribution of white rots (Watling 1982). In the Northern hemisphere, brown rot fungi appear to be adapted to conifers, since these decay fungi predominate in the primitive wood of gymnosperms (Gilbertson 1980; Ryvarde 1976, 1978). In contrast, in the Southern hemisphere, such fungi are also common in angiosperms (Rajchenberg 1989). However, there are proportionately fewer brown rot fungi in the tropics than in the Northern and Southern temperate regions (Quanten 1997; Buchanan 2001).

In brown rot the decomposition of cellulose and hemicellulose takes place at different stages. It is assumed that hydrogen peroxide is probably formed in a pre-cellulolytic phase, and easily penetrates into the cell wall and, together with iron ions, overcomes the lignocellulose matrix by oxidative

depolymerisation (Koenigs 1974a,b). This assumption seems necessary, as cellulose-degrading enzymes are relatively large and the much smaller cell-wall capillaries (>2 nm) cannot be simply penetrated by cellulolytic enzymes without loosening of the cell wall matrix (Cowling & Kirk 1976; Hill & Papadopoulos 2001). For this reason, cell-wall degradation occurs not in the immediate vicinity of the hyphal sheath (Green et al. 1989) or out from the lumen, but the ectoenzymes of the brown rot fungi must first diffuse very deeply into the cell wall through the S_3 layer, to degrade the cellulose-rich S_2 layer. Thus, high lignin content tends to delay the diffusion of the large molecules of the cellulose-degrading enzymes into the cell wall (Koenigs 1974a,b; Highley & Murmanis 1987).

Brown rots were examined microscopically in wood blocks of one conifer and four broadleaved trees: Norway spruce, birch (*Betula pendula*), oak, robinia and sycamore incubated with *Fomitopsis pinicola* and *Laetiporus sulphureus* and on beech incubated with *F. pinicola* (Schwarze et al. 2003). Most of the hyphae grew on the surface of the S_3 layer in the lumen (Fig 25). In oak wood incubated with *L. sulphureus*, the S_3 layer and compound middle lamellae were altered only slightly but the S_2 layer was extensively degraded (Fig 27). On the other hand, the Norway spruce wood colonized by *F. pinicola* showed extensive zones where cracks ran from the S_3 layer outwards through the secondary and primary cell wall but not into the

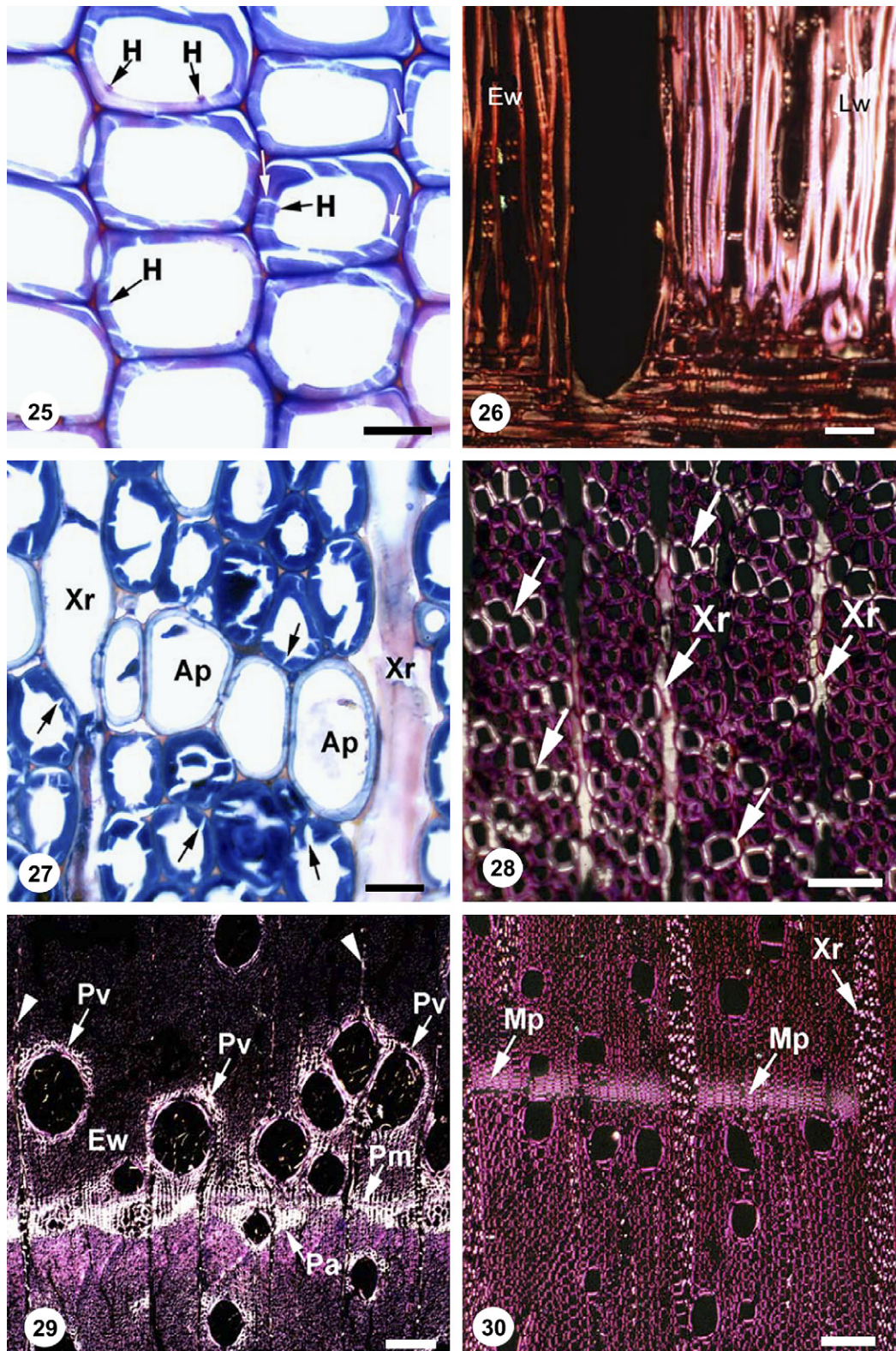


Fig. 25–30 – V. Brown rot. (25) T.S. of Norway spruce wood incubated with *Fomitopsis pinicola* showing numerous clefts (arrows) in the secondary walls of the tracheids. In close vicinity of hyphae (H) the secondary wall appears light reddish in colour due to degradation of hemicellulose and cellulose and staining of modified lignin with safranin. Bar, 10 μ m. (26) R.L.S. of beech wood incubated with *Fomitopsis pinicola* and viewed between crossed Nicols. Note loss of birefringence in the fibre-tracheids of the earlywood (Ew), whereas the cell walls in the latewood (Lw) appear bright. Bar, 50 μ m. (27) T.S. of oak wood incubated with *Laetiporus sulphureus*. The secondary walls of the libriform wood fibres show numerous clefts (arrows). In contrast, cell walls of the apotracheal-reticulate parenchyma (Ap) and xylem rays (Xr) show no signs of degradation. Xr,

middle lamella (Fig 25). The first evidence of cell wall degradation by brown rot fungi is the loss of birefringence (Schulze and Theden 1937; Wilcox, 1993; Schwarze 1995; Schwarze et al. 2004). This feature is superior to any other diagnostic approach and is readily imaged in the sections under polarized light. Although all the host/fungus combinations showed similar modes of cell wall degradation, they differed in intensity within various parts of the annual ring (Schwarze et al. 2003). This could be seen microscopically by placing the specimen between crossed Nicols, so that only regions containing intact cellulose were illuminated (Lohwag 1937; Schulze & Theden 1937). Regions where cellulose had been degraded appeared dark. The lignin and hemicellulose appeared dark irrespective of the state of decomposition, owing to their non-birefringent nature.

Observations of birefringence under polarized light showed that in all hosts both brown rot fungi affected cells of the earlywood before those of the latewood (Fig 26). A slow rate of diffusion may explain why tree species with naturally dense and highly lignified wood are relatively resistant to decay (Rayner & Boddy 1988; Schwarze et al. 2004). This may be particularly important in resistance to brown rot, which mainly involves the diffusion of degradative substances, rather than the direct erosion of cell walls by fungal hyphae.

After 6 and 12 weeks incubation there was preferential decomposition of cellulose in the cell wall of libriform-wood fibres and fibre tracheids, but not in axial parenchyma, xylem ray parenchyma nor latewood fibres (Figs 28–30). Resistance of latewood fibres appears to be related to a higher degree of cell wall lignification, which hampers diffusion of cellulolytic enzymes into the cell wall, thus resulting in a greater resistance towards decomposition (Schwarze et al. 2003). Preferential decomposition of certain zones of the annual ring was also seen in oak, robinia and sycamore wood incubated with *L. sulphureus* (Figs 28–30). Decomposition of cellulose, as shown by the loss of birefringence, occurred at an early stage in the early wood fibres, but the cell walls of the nearby axial parenchyma cells showed no sign of degradation (Schwarze 1995). In robinia these cells form prominent aggregations within the wood, both at the beginning of growth rings (apotracheal-terminal) and near to vessels of the earlywood (paratracheal-vasicentric) (Fig 29).

Resistance of parenchyma cells to decomposition by brown rot fungi

Interestingly, weight losses from different wood species appeared to correlate with the content of parenchyma cells

found in the xylem (Fig 31). Thus, the highest weight losses recorded were associated with Norway spruce and birch wood, which have a low parenchyma content of 5–10% (Wagenführ 1999), whereas the lowest weight losses were associated with oak and robinia wood, which have a high parenchyma content of 35–40% (Table 2). By contrast, moderate weight losses were associated with sycamore wood, which has a moderate parenchyma cell content of 16–20% (Wagenführ 1999).

To test the hypothesis that parenchyma cells are resistant to brown rot fungi we determined the decay rate of balsa sapwood, which consists of 92% parenchyma cells, with a range of decay fungi: one white rot fungus *Trametes versicolor*, and four brown rot fungi *Poria placenta*, *Gloeophyllum trabeum*, *Coniophora puteana* and *Fomitopsis pinicola*. For assessment of fungal vigour, Scots pine wood blocks were also incubated with each fungal strains.

Although wood blocks were strongly colonized by hyphae of all brown rot fungi after six and twelve week's incubation, weight losses of *P. placenta*, *G. trabeum* and *C. puteana* were negligible. *Poria placenta* and *C. puteana* even caused a slight increase in dry weight due to the large amount of mycelium within the wood (Fig 32). All wood decay fungi caused high weight losses in Scots pine wood blocks, indicating that the isolates were vigorous and that resistance of balsa wood was not related to the use of degenerated fungal isolates. In contrast to all other species the obligatory strains used for European standard EN 113 (1997), *F. pinicola* caused significant dry weight losses in both balsa and Scots pine wood blocks (Fig 32). Not surprisingly, the white rot fungus *T. versicolor* caused higher dry weight losses in wood of balsa than in Scots pine (Fig 32) as white rot fungi preferentially degrade parenchyma cells.

Various studies have emphasized the resistance of vessel cell walls to decomposition by white rot fungi (Blanchette et al. 1988). The resistance of vessels to decomposition appears to be related to their high lignin: carbohydrate ratio, lignin monomer composition and cell wall morphology. Earlier studies provide sound evidence that the resistance of parenchyma cells to decomposition by brown rot fungi is not associated with the lignin composition or the total lignin content within parenchyma cells. UV-microscopy of parenchyma and fibre tracheids and libriform wood fibre in oak and sycamore wood illustrated that there are only negligible differences in their lignin composition (Schwarze et al. 2000a,b; Baum 2001). Thus, it appears that the cell wall morphology rather than the lignin composition or total amount of lignin is responsible for the higher resistance of parenchyma cells to decomposition by brown rot fungi.

xylem ray. Bar, 20 μm . (28) T.S. (27) viewed between crossed Nicols. Birefringence is apparent from the cell walls of the apotracheal-reticulate parenchyma (arrows) and the xylem rays (Xr). Degraded cell walls of the libriform wood fibres appear darker and show a weak birefringence. Bar, 50 μm . (29) T.S. of robinia wood incubated with *Laetiporus sulphureus* and viewed between crossed Nicols. Birefringence is apparent from prominent aggregations of parenchyma cells in the early wood. Apotracheal-marginal (Am), paratracheal-vasicentric (Pv), paratracheal-aliform (Pa), and xylem ray parenchyma (pointers). Degraded cell walls of the libriform wood fibres in the earlywood (Ew) are dark and show no birefringence. Bar, 60 μm . (30) T.S. of sycamore wood incubated with *Laetiporus sulphureus* and viewed between crossed Nicols. Birefringence is apparent at the border of the annual ring as band from the apotracheal-marginal parenchyma cells (Mp) and the xylem rays (Xr). Bar, 50 μm . Figs 25–30 reproduced from Schwarze et al. (2003) by permission of the Deutsche Gesellschaft für Mykologie.

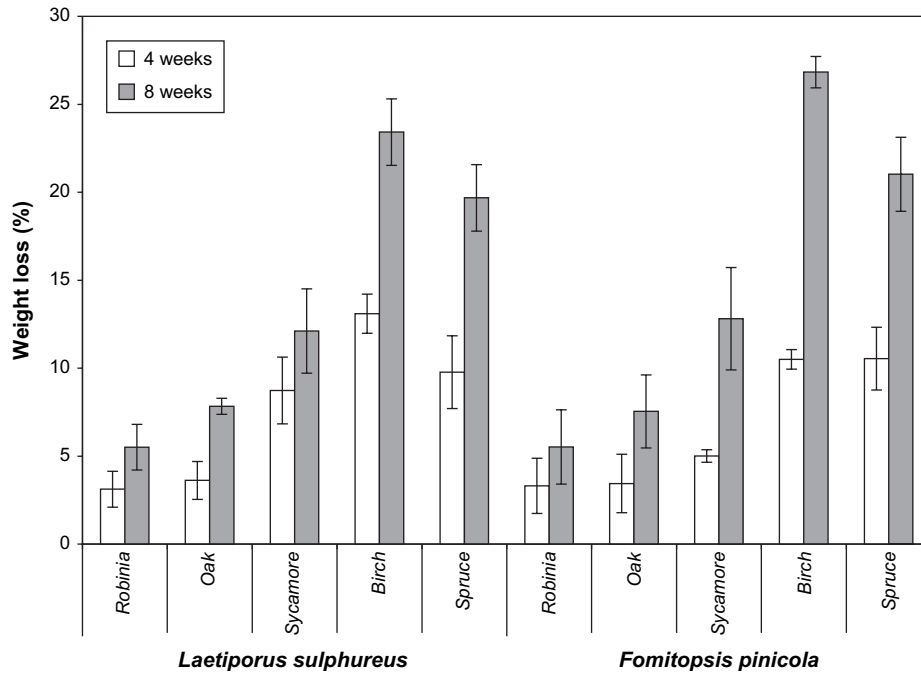


Fig. 31 – Brown rot. Dry weight losses of sapwood blocks (%) incubated with *Laetiporus sulphureus* and *Fomitopsis pinicola* after 6 and 12 weeks incubation (n = 10). Bars, SE. Reproduced from Schwarze et al. (2000a) by permission of the Deutsche Gesellschaft für Mykologie.

Previous studies have demonstrated that decay resistance of hardwoods is greater to brown rot than to white rot. Studies on the natural resistance of 17 species of angiosperms and 3 species of conifers to the brown rot basidiomycete *Oligoporus placenta* and two white rot species *T. versicolor* and *Ganoderma applanatum* showed that gymnospermous species were more susceptible to the brown rot than the white rot fungi (Sukartana & Highley 1997). Seven angiospermous species native to

Taiwan, were decayed more heavily by *T. versicolor* than by the brown rot fungus *L. sulphureus* (Wang-CheLan et al. 1999). In trunks of Canary Island date palm (*Phoenix canariensis*), which possess a high parenchyma content, white rot fungi caused significantly more weight loss (63%) than brown rot fungi (32%) (Adaskaveg et al. 1991).

These studies did not examine whether parenchyma cells were resistant to decomposition by brown rot fungi and

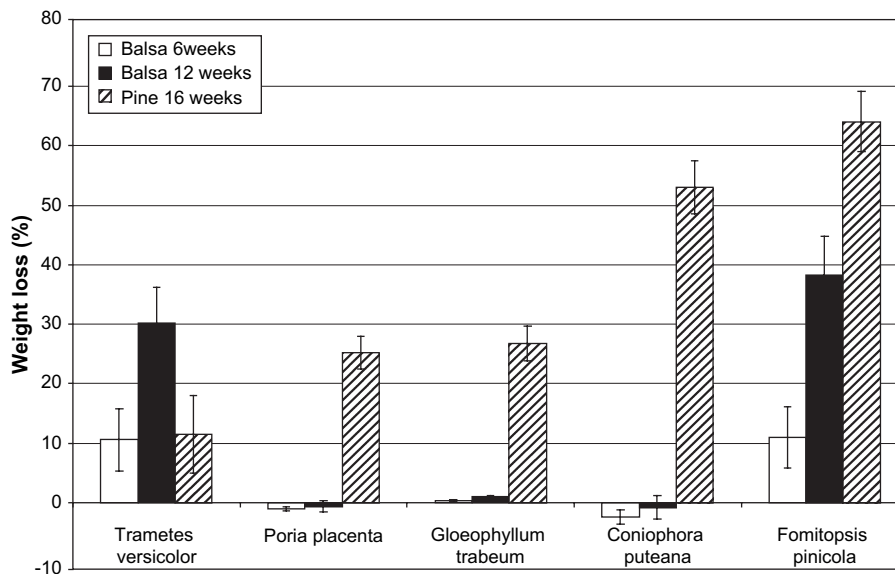


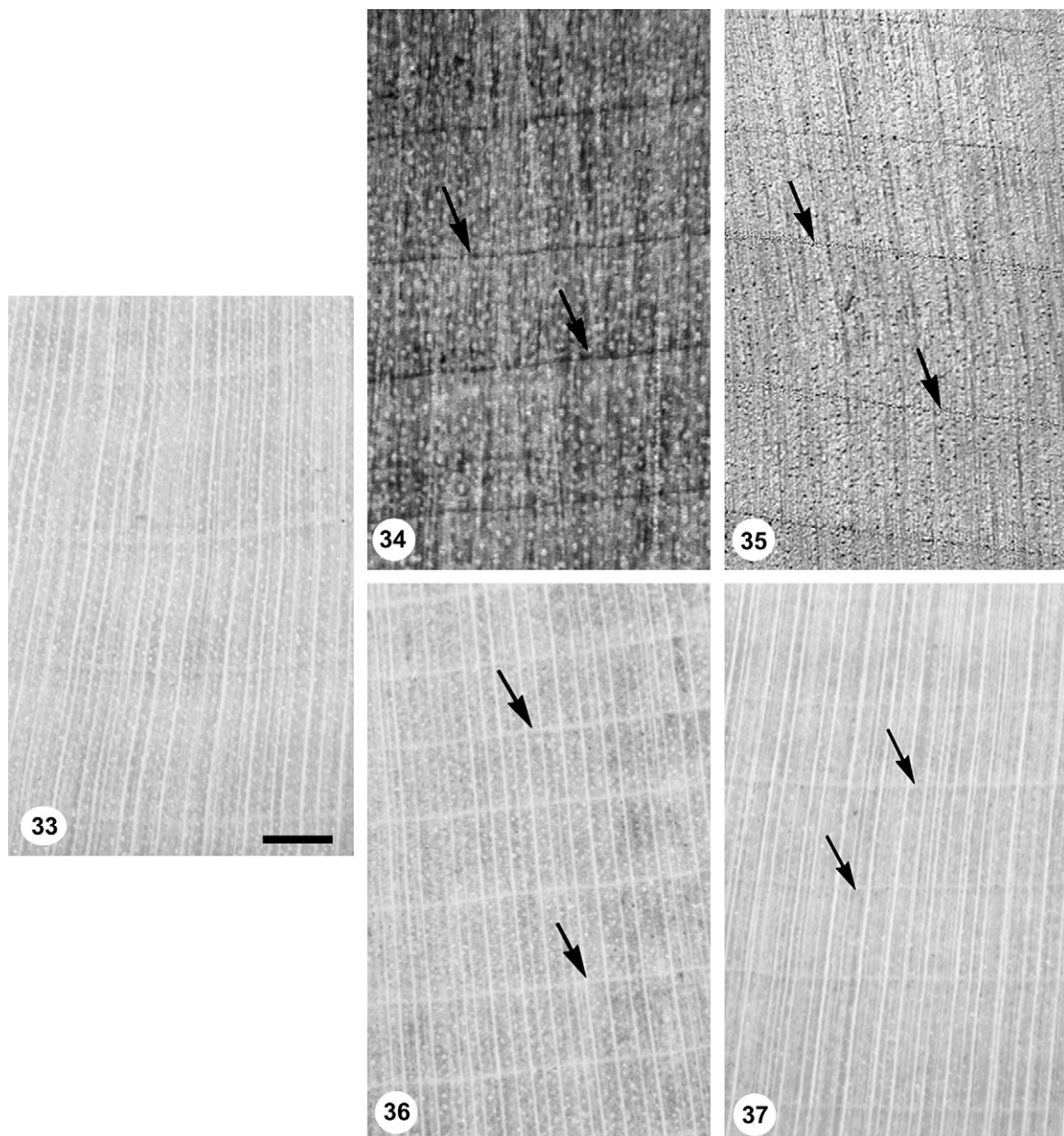
Fig. 32 – Brown rot. Dry weight losses of sapwood blocks (%) of balsa and Scots pine incubated with one white rot fungus *Trametes versicolor* and three brown rot fungi *Poria placenta*, *Gloeophyllum trabeum*, and *Coniophora puteana* after 6 and 12 weeks incubation (n = 10). Bars, SE.

results may simply reflect a low co-evolutionary adaptation of brown rot fungi to hosts with a high parenchyma content such as in the xylem of highly evolved angiospermous trees (Braun 1970). Additional evidence is provided by the fact that many white rot fungi are predominantly associated with angiosperms and preferentially degrade parenchyma cells during early stages of decay (Schwarze & Fink 1998).

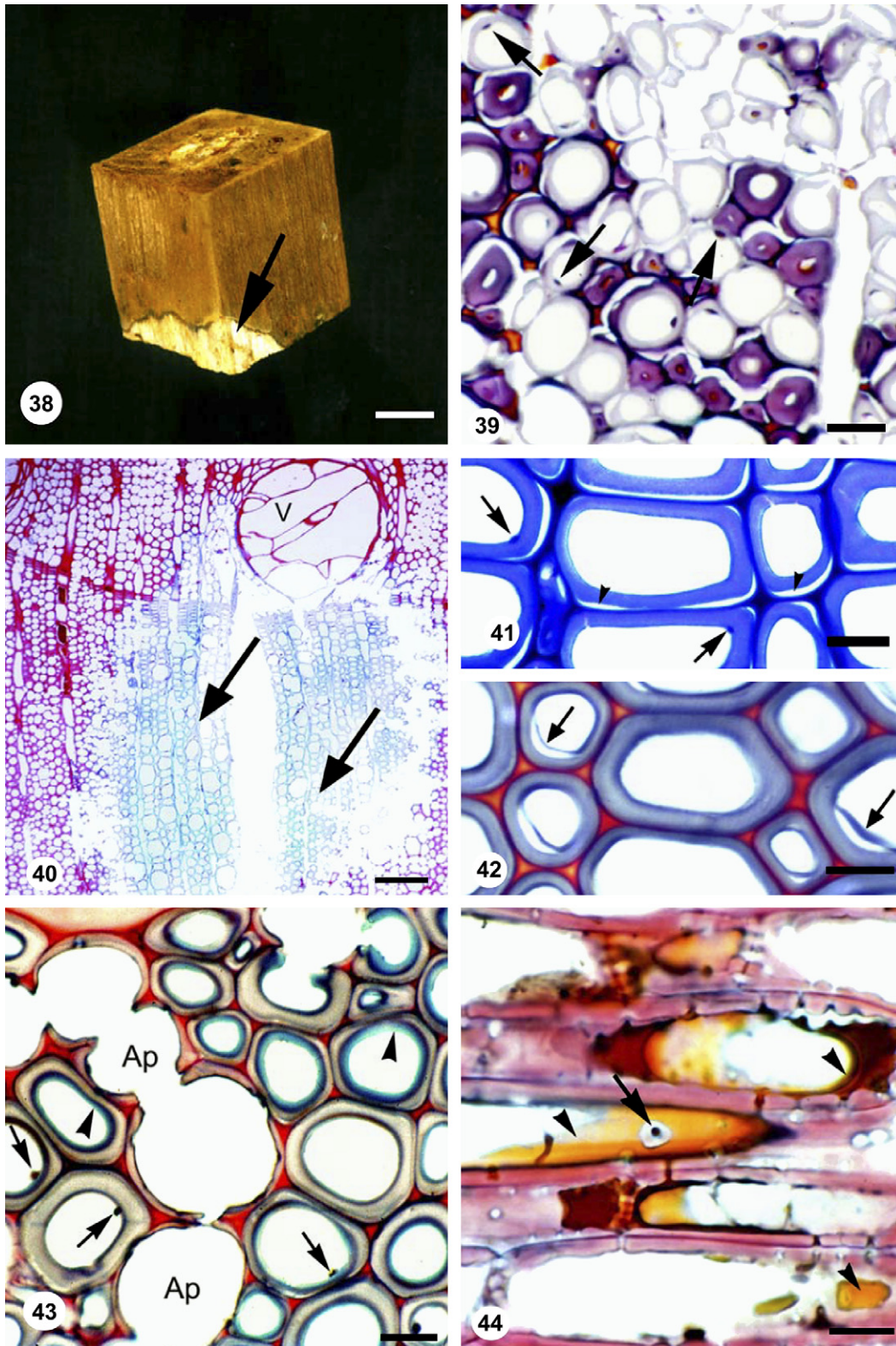
Brown rot fungi as tools for tree ring analysis

The fact that parenchyma cells are resistant to decomposition by brown rot fungi, can be used to facilitate detection of

annual rings in wood for dendrochronological studies. Thus, a typical anatomical feature of the wood of many trees either is the presence of apotracheal terminal parenchyma or strongly lignified latewood fibres at the border of growth rings. Several dendrochronological methods have been developed to improve the detection of annual rings (Schweingruber 1978; Cook & Kairiukstis 1990; Schweingruber 2001). Despite extensive studies, recognition of tree ring structures within diffuse porous and light coloured wood is still a major challenge for dendrochronology (Fig 33; Schweingruber 2001). Brown rot fungi have been employed as analytic tools for enhancing the appearance of tree rings in diffuse porous wood (Deflorio



Figs. 33–37 – VI. Tree rings. T.S. of sycamore wood samples either treated with traditional preparation methods to enhance visibility of tree rings or incubated with brown-rot fungi. (33) Sample treated with a diamond flycutter (reference). (34) *Fomitopsis pinicola*, 33% weight loss. (35) *Laetiporus sulphureus* 5.2 % weight loss. (36) Sample treated with a diamond flycutter than stained. (37) Sample treated with a diamond flycutter and then with crystal clear varnish. Arrows: annual ring borders. Bar, 10 mm. Figs 33–37 reproduced from Deflorio et al. (2005) by permission of Elsevier.



Figs. 38–44 – VII. White rot selective delignification. (38) Wood block of robinia artificially incubated with *Perenniporia fraxinea*. Note early stages of selective delignification (arrow) commencing from the bottom of the wood block that was exposed to the fungus. Bar, 2 cm. (39) T.S. of robinia wood incubated with *Perenniporia fraxinea*. Delignification by hyphae (arrows) growing within the cell lumen of parenchyma cells is apparent in the earlywood. Note libriform-wood fibres resist degradation. Bar, 10 μ m. (40) T.S. stained with safranin and astra-blue showing white pocket rot in oak wood by *Grifola frondosa*. Note delignified, light-blue cell regions (arrows) are surrounded by intact cell regions stained red. Bar, 50 μ m. (41) T.S. showing selective delignification of Norway spruce wood by *Heterobasidion annosum*. Degradation of middle lamellae by

et al. 2005). Exposure of annual ring borders in degraded sycamore and birch wood samples, treated with brown rot basidiomycetes fungi *Fomitopsis pinicola* and *Laetiporus sulphureus*, was superior to conventional treatment methods ((Deflorio et al. 2005; Figs 33–37).

6. White rot

White rots are caused by basidiomycetes and by certain ascomycetes. The common feature of all these fungi is that they can degrade lignin as well as cellulose and hemicelluloses. However, the relative rates of decomposition of lignin and cellulose vary greatly according to the species of fungi and the conditions within the wood (Fig 11). As with brown rots, there is additional variation related to the preferential decay of different zones within the annual ring.

Selective delignification

In selective delignification, lignin is degraded earlier in the decay process than cellulose or hemicellulose. The hyphae grow in the cell lumina in some cases, so that the lignin is dissolved out of the adjacent cell wall. In other cases, hyphae penetrate the cell walls and initially delignify the middle lamella so that the cells tend to separate. Cellulose was left relatively unaltered during selective delignification, at least in the early stages of decay (Schwarze 1995; Schwarze et al. 2004).

In contrast to brown rot, parenchyma cells are often preferentially degraded by white rot fungi that cause a selective delignification. Light microscopy studies on robinia wood artificially incubated with *Perenniporia fraxinea* revealed selective delignification in moister parts of the wood block (Fig 38). Hyphae were apparent within the cell lumen of parenchyma of the earlywood. In contrast to brown rot, parenchyma cells were preferentially degraded during early stages of delignification, whereas libriform-wood fibres, which are rapidly degraded by brown rot fungi, were resistant to decomposition (Fig 39).

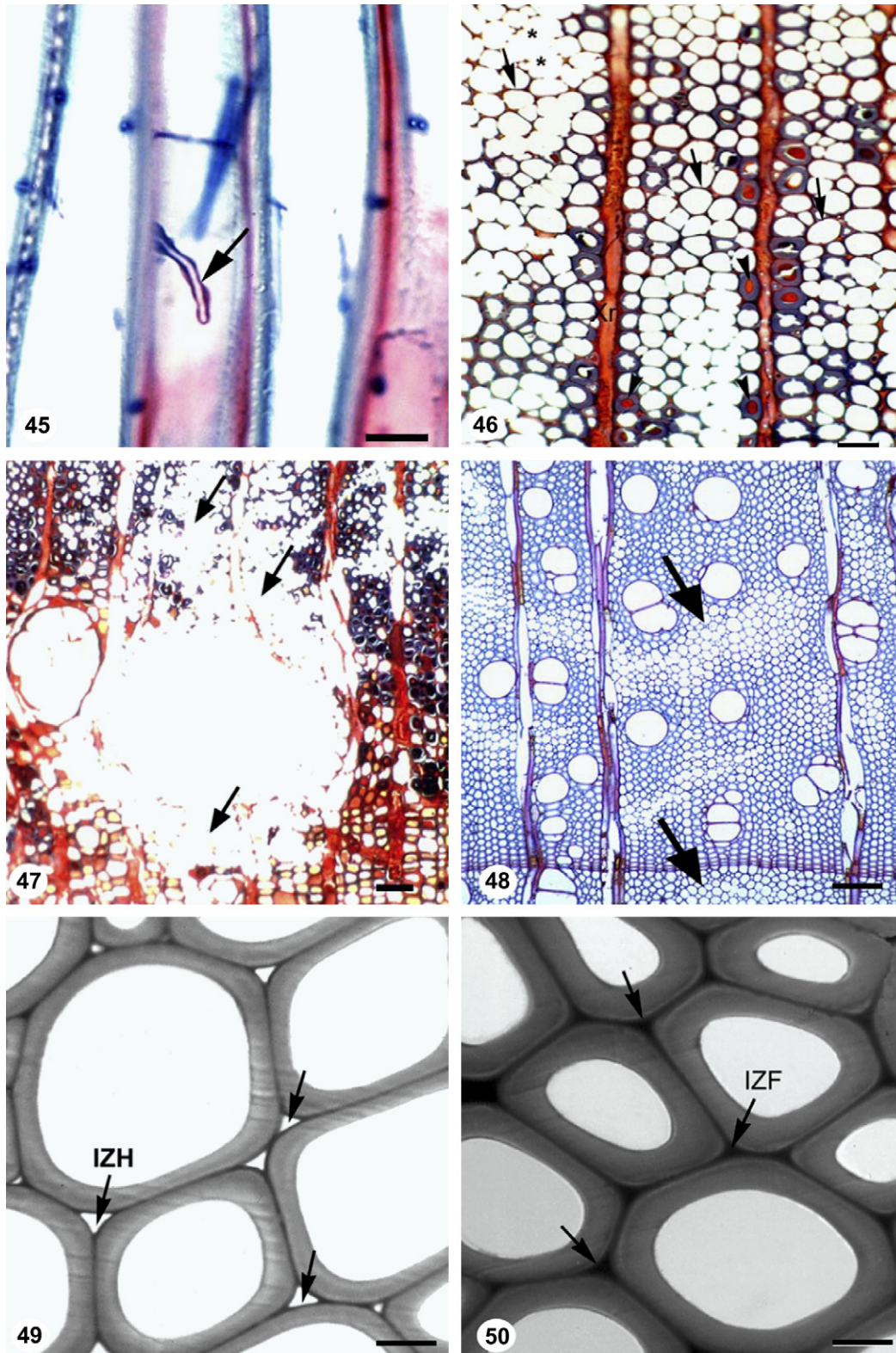
Some of the fungi that cause selective delignification tend to do so in discrete pockets which show up paler than the surrounding wood due to the high concentrations of cellulose that remain within them (Hartig 1878; Blanchette 1980). Examples of such a white pocket rot are caused by *Phellinus pini* in pine and *Grifola frondosa* in pedunculate oak (Fig 40). High resolution microscopy of decay of Norway spruce wood by *Heterobasidion annosum* showed typical delignification within the compound middle lamella between tracheids (Schwarze 1995), confirming earlier findings by (Blanchette 1984a,b).

Interestingly the middle lamella was delignified in the virtual absence of any alteration in the primary and secondary walls of tracheids (Schwarze 1995). This pattern of early decay resulted in the separation of single tracheids from each other when viewed in transverse section (Fig 41). Preferential decomposition also occurred on a larger scale, since the above alterations began in the latewood and developed subsequently in the earlywood. By contrast, complete delignification of xylem ray parenchyma was followed by complete decomposition of the secondary walls.

Scots pine wood was also selectively delignified by *H. annosum*, but in Norway spruce this occurred from within the lumina of the latewood tracheids (Fig 42) and not in the middle lamellae. Single sheaths of the inner secondary wall became detached and peeled off inwards towards the lumina, as previously described by Peek et al. (1972). These separated sheaths continued to show birefringence at this stage, indicating the presence of intact cellulose (Schwarze 1995). *Ganoderma pfeifferi* also caused a selective delignification in beech and oak wood (Schwarze 1995), with preferential degradation of the middle lamellae in the latewood and the xylem rays. On the other hand, a simultaneous rot was the predominant type of decay throughout the wood, highlighting the fact that not only can selective delignification and simultaneous rot both be caused by a single fungus, but that the two processes often occur side by side (Blanchette 1980).

Although cellulose decomposition is a common sequel to selective delignification, there are extreme cases where lignin can be decomposed throughout a large volume of wood, while the cellulose remained almost unaffected (Blanchette 1984a,b; Adaskaveg & Gilbertson 1986; Adaskaveg et al. 1990; Leatham et al. 1990). In the temperate rainforests of southern Chile, Phillipi (1893) described a type of decayed wood called "palo podrido" which consists of 97 % cellulose and only 0.9 % lignin (González et al. 1986; Agosin et al. 1990). Palo podrido is a general term for white rot decay that is either selective or nonselective for the removal of lignin, whereas palo blanco describes the white decayed wood that has advanced stages of delignification. Selective delignification occurs mainly in trunks of *Eucryphia cordifolia* and *Nothofagus dombeyi*, which have a low lignin content and whose lignins have the largest amount of β -aryl ether bonds and a high syringyl/guaiacyl ratio (Agosin et al. 1990). A *Ganoderma* species was the main white rot fungus associated with the decay. The structural changes in lignin during white rot examined by thioacidolysis, revealed that the β -aryl ether-linked syringyl units were more specifically degraded than the guaiacyl ones, particularly in the case of selective delignification. Ultra structural studies showed that lignin was first removed from the secondary wall nearest the lumen and then throughout the secondary

hyphae (arrows) growing in the cell lumen results in separation (pointers) of tracheids. Bar, 10 μ m. (42) T.S. of Scots pine wood incubated with *Heterobasidion annosum*. Selective delignification (pointers) of tracheids results in delamination (arrows) within the secondary wall. Bar, 10 μ m. (43) T.S. of beech wood incubated with *Ganoderma adspersum*. Note early stages of delignification in secondary walls by hyphae (arrows) growing in the cell lumen of fibre-tracheids results in a distinct colour change. Ap, axial parenchyma. Bar, 10 μ m. (44) R.L.S. of a reaction zone in beech wood naturally infected with *Ganoderma adspersum*. Brownish polyphenols (pointers) are apparent within the lumina of xylem ray parenchyma. Note hypha (arrow) penetrating polyphenols (pointers). Bar, 10 μ m. Figs 40–44 reproduced from Schwarze et al. (2004) by permission of the Rombach Verlag.



Figs. 45–50 – VIII. Simultaneous rot. (45) T.L.S. beech wood naturally infected with *Fomes fomentarius*. Cell wall erosion (arrow) is apparent in close vicinity to a hypha growing on the S₃-layer within the cell lumen. Pointers, bore holes. Bar, 10 μm. (46) T.S. of beech wood naturally infected with *Fomes fomentarius*. Progressive cell wall thinning of the fibre-tracheids (arrows) occurs from the lumen outwards. Note fibre tracheids containing polyphenols resist degradation (pointers). Xr, xylem ray. asterix, completely degraded cell regions. Bar, 50 μm. (47) T.L.S. of oak wood naturally infected with *Fomes fomentarius*. Note preferential degradation of fibre tracheids (arrows) surrounding vessels of the earlywood. Bar, 50 μm. Figs 45–47 reproduced from Schwarze et al. (2004) by permission of the Rombach Verlag. (48) T.S. of sycamore wood incubated with

wall toward the middle lamella. The middle lamella and cell corners were the last areas to be degraded.

Selective delignification is well documented for *Ganoderma* spp. and corresponds with their ability to degrade polyphenols preferentially, as these are chemically similar to lignin (Fig 43). The extraordinary preferential development of *G. adspersum* within an environment rich in polyphenols may be related to an ability to respond to certain chemical stimuli. Some volatiles stimulate not only the overall growth of *Ganoderma* spp., but also their direction of growth towards the source (Rayner and Boddy 1988; Schwarze & Ferner 2003). Thus *G. adspersum* degraded the occlusions in the lumina of axial parenchyma and fibre tracheids to defeat reaction zones, both in the standing tree and in artificially incubated wood blocks (Fig 44).

Simultaneous rot

Many species cause simultaneous rot (Fig 12) in angiosperms, but only rarely in gymnospermous wood. This selection may be related to the extremely resilient S₃ layer of tracheids that hampers degradation by hyphae from within the cell lumen outwards. By contrast, low molecular weight substances causing brown rot and selective delignification, simply diffuse through the S₃-layer of the secondary wall.

In simultaneous rot, decomposition takes place close to the hyphae involved, and results in the formation of erosion troughs where they grow on the cell wall (Figs 45 and 46). The enzymes that they secrete are able to decompose all substances of the lignified cell wall (Liese 1970). As the decomposition of cellulose, hemicellulose, and lignin occurs at nearly the same rate, the term is appropriate, although the general term white rot is often applied. The coalescence of the erosion troughs induced by numerous hyphae results in a general cell wall thinning from the lumen outwards (Fig 46) (Liese 1970; Schwarze 1995).

Fomes fomentarius causes a typical simultaneous rot. In beech, the hyphae were very abundant and colonised the wood via xylem rays and vessels, following the pathways of least resistance (Rayner & Boddy 1988). However, the fungus entered adjacent fibre tracheids mainly via micro hyphae, which penetrated the walls horizontally (Schwarze et al. 2004). Following penetration of fibre tracheids and libriform wood-fibres of beech and oak, *F. fomentarius* caused a general thinning of the cell walls due to the combined erosive effect of the hyphae growing on the S₃ layer (Figs 45 and 46). These hyphae, were apparent in transverse sections, which also showed that cell wall thinning was hampered by the highly lignified middle lamellae and the cell wall corners (Fig 45). These regions were degraded only slowly in the final stages of decay. Apart from showing the uneven rate of cell wall thinning, *F. fomentarius* did not show any preferential attack on specific cell types or wall layer in beech. It was, however,

apparent in oak wood that fibre tracheids in close proximity to vessels were preferentially degraded (Fig 47; Schwarze et al. 2004).

Effect of lignin content in cells of *Acer pseudoplatanus* on decomposition by white rot fungi

Preferential decomposition by white rot fungi is often followed by more general utilization of the wood, but some species show only a limited ability to degrade certain cell types or cell wall constituents. In particular, there are some species which leave the vessels of angiosperm trees largely undegraded, even at a relatively advanced stage of decay (Blanchette et al. 1988). This is apparently due to the high lignin: carbohydrate ratio of vessel walls, together with their morphology and the monomeric composition of their lignin (Blanchette et al. 1988). *Armillaria mellea* is classified as a white rot fungus, based on its lignolytic ability, although chemical analysis has shown this to be rather low at early stages of decomposition when compared with many other white rot fungi (Campbell 1931, 1932). Preliminary studies of inoculated wood blocks of sycamore showed a preferential degradation of certain cell wall regions by *A. mellea* (Schwarze et al. 2000b).

Microscopical observations revealed a very distinctive pattern of degradation in wood of sycamore under both natural and artificial conditions (Schwarze et al. 2000b). Decomposition began preferentially within groups of libriform wood fibres containing intercellular spaces, whereas fibre regions lacking such spaces, vessels and axial parenchyma were undegraded and remained largely intact even when decay had become advanced elsewhere (Figs 48–50; Schwarze et al. 2000b). Semiquantative analysis with UV-microscopy showed a correlation between UV-absorbance and the visually assessed degradation pattern which indicated differences in the degree of lignification in different cell types (Schwarze et al. 2000b; Figs 49 and 50).

The vessels, unlike the two types of fibre and axial parenchyma, showed a high proportion of guaiacyl lignin within their secondary walls, as indicated by their peak UV-absorbance at 280 nm (Schwarze et al. 2000b; Fig 51). This interpretation was supported by the low absorbance of visible light at 520 nm after staining for Mäule colour reaction, as this appears to be characteristic of a low syringyl monomer content (Wu et al. 1990). This is also in good agreement with Ferguson and Goring (1970b), who showed a high proportion of guaiacyl lignin in the vessel cell walls of a range of angiosperms. Interestingly, these observations are also in good agreement with chemical analysis by Campbell (1931, 1932), which showed that the lignolytic ability of *A. mellea* s. lat. is low at early stages of decomposition, compared to that of many other white rot fungi. Thus, although *A. mellea* has the capacity to degrade all cell wall constituents, it preferentially degraded cell walls with relatively low lignin content. The two types of fibre

***Armillaria mellea*. At an early stage of decay, fibre regions in between vessels (arrows) containing intercellular spaces (IZH) are preferentially degraded, whereas fibre regions without intercellular spaces (IZF) surrounding vessels are resistant to decay. Bar, 50 µm. (49) T.S. of sound libriform-wood fibre of sycamore: UV-micrograph at 280 nm (bars, 10 µm). Note the relatively low UV-absorption of the fibre secondary wall within areas containing intercellular spaces (IZH, arrows) is apparent from their light colour. Bar, 20 µm. (50) The fibre secondary walls within areas without intercellular spaces (IZF, arrows) appear dark, indicating a higher UV-absorption compared with the fibres in Fig 49 Bar, 20 µm.**

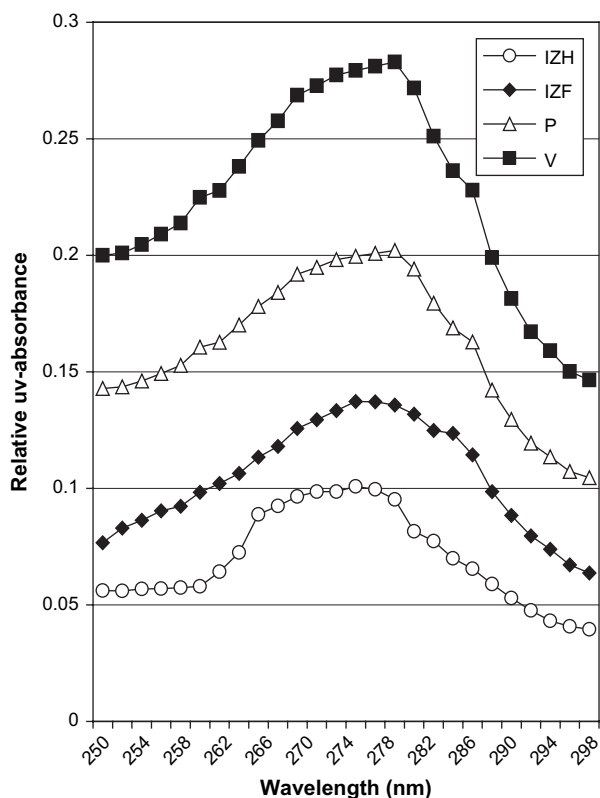


Fig. 51 – Simultaneous rot. uv-absorbance in the secondary walls of sound sycamore cells showing a peak at 280 nm for vessels (v) and peaks at 276 nm for axial parenchyma (P), fibres with intercellular spaces (IZH) and without intercellular spaces (IZF). Figs 48–51 Reproduced from Schwarze et al. (2000b) by permission of the British Mycological Society.

region differed not only in the presence of intercellular spaces, and hence in the potential for gas exchange, but also in their degree of lignification. This was higher in the more resistant type, as shown by staining of undecayed wood with toluidine blue-O, by microspectrometry after staining for the Mäule colour reaction, and by UV-microscopy (Schwarze et al. 2000b). A spatially similar pattern of cellulose decomposition was induced by the brown rot basidiomycete *L. sulphureus*.

Angiosperm wood containing guaiacyl-rich lignin is more resistant to decay than that containing syringyl-rich lignin (Syafii & Yoshimoto 1991). Although the monomeric composition may play a part in the decay resistance of vessels, the two types of fibre regions observed in the *A. mellea* study appeared to have similar forms of lignin in their secondary walls (Fig 51; Schwarze et al. 2000b). Thus lignin concentration is more likely than lignin composition to play a role in the decay resistance of the fibres without intercellular spaces. Even though locally high lignin concentrations probably have a role in determining the pattern of decay induced by *A. mellea* in wood of sycamore, it is important also to consider the possible influence of accompanying differences in aeration of the tissue. A high moisture content and low availability of oxygen restrict wood decomposition processes and therefore act as a passive microenvironmental form of defence within intact sapwood

(Boddy & Rayner 1983; Pearce 1996). In this context, one prominent feature of fibre regions in sycamore wood degraded by *A. mellea* is the presence of abundant intercellular spaces. It is conceivable that the gaseous microenvironment in these regions is more favourable for decomposition by *A. mellea* than in the regions without intercellular spaces.

7. Soft rot

The work of Savory (1954) marked an important further step in the understanding of decomposition processes by lignolytic fungi. His description of decay by ascomycete and deuteromycete fungi revealed a particular pattern formerly observed by Bailey and Vestal (1937), Tamblin (1937) and Baghoun and Linder (1944) in wood. This type of decay was termed 'soft rot' because of the spongy texture of the wood surface. Soft rots differ from both brown and white rots in their pattern of development, which involves a process of hyphal tunnelling inside the lignified cell walls (Fig 13). Soft rot was therefore described as distinct from brown- and white rot forms of wood decay normally attributed to lignolytic basidiomycetes and some of the larger ascomycetes. Soft rots are chemically more similar to brown rots than white rots since cellulose and hemicellulose is decomposed while lignin is modified slightly. Another common feature of brown and soft rot is the demethylation of methoxyl groups.

On the basis of polyphenol activity, certain fungi, including members of the genera *Daldinia*, *Hypoxylon*, *Kretzschmaria* and *Xylaria* were previously considered to be white rot fungi, but light microscopy studies show that their mode of degradation is more correctly classified a Type 1 or Type 2 form of soft rot (Nilsson et al. 1989; Blanchette et al. 1990; Anagnost 1998; Schwarze et al. 2004). Soft-rot occurs in damp or fluctuating environments, and is common in fence posts, telegraph poles, the timbers of cooling towers, and wood in aquatic environments. The fungi that cause soft rots include Ascomycota and mitosporic species, such as *Chaetomium globosum*, *Hemicella grisea*, *Petriella setifera*, *Phialophora mutabilis*, *Trichurus spiralis* in terrestrial environments and species of *Lulworthia*, *Halosphaeria* and *Pleospora* in marine and estuarine environments (Baghoun & Linder 1944; Nilsson 1973).

The distinguishing feature of soft rots is the formation of cavities within the S_2 layer of the secondary wall (Type 1) (Fig 52; Schacht 1863; Savory 1954; Findlay & Savory 1954; Cartwright & Findlay 1958). Soft rot cavities are initiated by fine penetration hyphae formed from hyphae in the lumina of wood cell walls. The penetration hypha grows through the innermost S_3 layer of the cell wall to the cellulose-rich S_2 where it either branches (T branches or L-bending) or grows axially within the cell wall following the orientation of the cellulose microfibrils (Fig 53). Fine hyphae that exhibit branching continue hyphal extension for a short time, but then cease apical growth (Hale & Eaton 1985a,b). At this stage a cavity is formed within the secondary wall around the fine hypha, which increases in diameter as the cavity develops (Fig 53). This is then followed by a further phase of apical growth at the hyphal tip, producing a needle like proboscis hypha (Hale & Eaton 1985a,b). This process repeats itself many times, leading to the formation of a spiral chain of cavities within the

wood cell wall, all orientated to the angle of the cellulose microfibrils and each showing different stages of cavity expansion (Fig 53). The repetitive start and stop pattern of apical hyphal growth results in gradual breakdown of the wood cell wall layer of the secondary wall (Type 1 degradation) and is the characteristic mechanism of cell wall degradation (Hale & Eaton 1985a,b). Discrete notches of cell-wall erosion by hyphae growing within the lumina, in addition to cavities formed by hyphae within the cell wall, are also frequently found in wood degraded by soft rot fungi. These erosion troughs (Type 2), which are indistinguishable from those of white rot fungi, have been attributed to a category of soft rot known as Type 2 attack (Fig 54; Corbett 1965; Hale & Eaton 1985a,b).

Traditionally, soft rot has been attributed to deuteromycetes and ascomycetes but not to basidiomycetes. Until recently only the latter were considered to occur deep within large volumes of decayed wood of standing trees (Blanchette 1992). Fungi commonly associated with soft rot are active only in the outer layers of wood, although they can progress inwards as the surface layers become eroded. In living trees, the most significant role so far attributed to soft rot fungi has been the decay of the bases of dead branches, which results in a form of natural pruning (Butin & Kowalski 1983, 1992). Increasing evidence also indicates that a range of brown- or white rot fungi cause a soft rot in addition or alternatively to their more typical mode of degradation (Table 3).

Soft rot in living trees caused by an ascomycete

Xylariaceae ascomycetes, e.g. *Daldinia concentrica* and *Hypoxylon* spp., cause decay in standing trees. *Kretzschmaria deusta* is exceptional in causing deep-seated and extensive decay in large volumes of wood and cause a soft rot (Schwarze et al. 1995b; Schwarze et al. 2004). It causes a distinctive pattern of decay, with many fine dark pseudosclerotial plates (Wilkins 1936; 1939a, 1939b, 1943; Gibbs & Greig 1990; Schwarze et al. 2004), and has been generally classified as a white rot. However, depending on the host and its lignin composition of the cell wall, *K. deusta* causes a soft rot Type 1 or 2 (Figs 52–54) (Schwarze et al. 1995b; Schwarze et al. 2004).

The presence of soft rot decay caused by *K. deusta* was revealed by high-resolution light microscopy in beech and large-leaved lime. The cell walls show hyphal tunnelling along the cellulose microfibrils of the S_2 layer, resulting in the appearance of holes in transverse sections (Figs 52 and 53). This mode of attack is typical of a Type 1 soft rot (Corbett 1965). Even at advanced stages, the persistence of a 'lignin-rich' skeleton, representing the most lignified components of the wood, preserves stiffness so that the wood becomes brittle (Schwarze et al. 1995b; Schwarze et al. 2004). The persistence of lignin-rich regions of the wood matrix in trees decayed by *K. deusta* is not only due to their high lignin content *per se*, but also to their high percentage of guaiacyl lignin. Members of the Xylariaceae have relatively poor lignolytic ability, and this is mainly confined to the decomposition of syringyl lignin (Nilsson et al. 1989). In wood that has a high percentage of syringyl lignin, e.g. sycamore, members of the xylariaceous cause a Type 2 soft rot reminiscent of a simultaneous rot (Fig 54). The ability of *K. deusta* to function as a soft rot fungus in living trees is of considerable interest, since this type of

decay has not been previously thought to occur within standing trees. On the other hand, this is not very surprising, since this fungus is an ascomycete, like many of the fungi that cause superficial soft rots in felled and fallen wood.

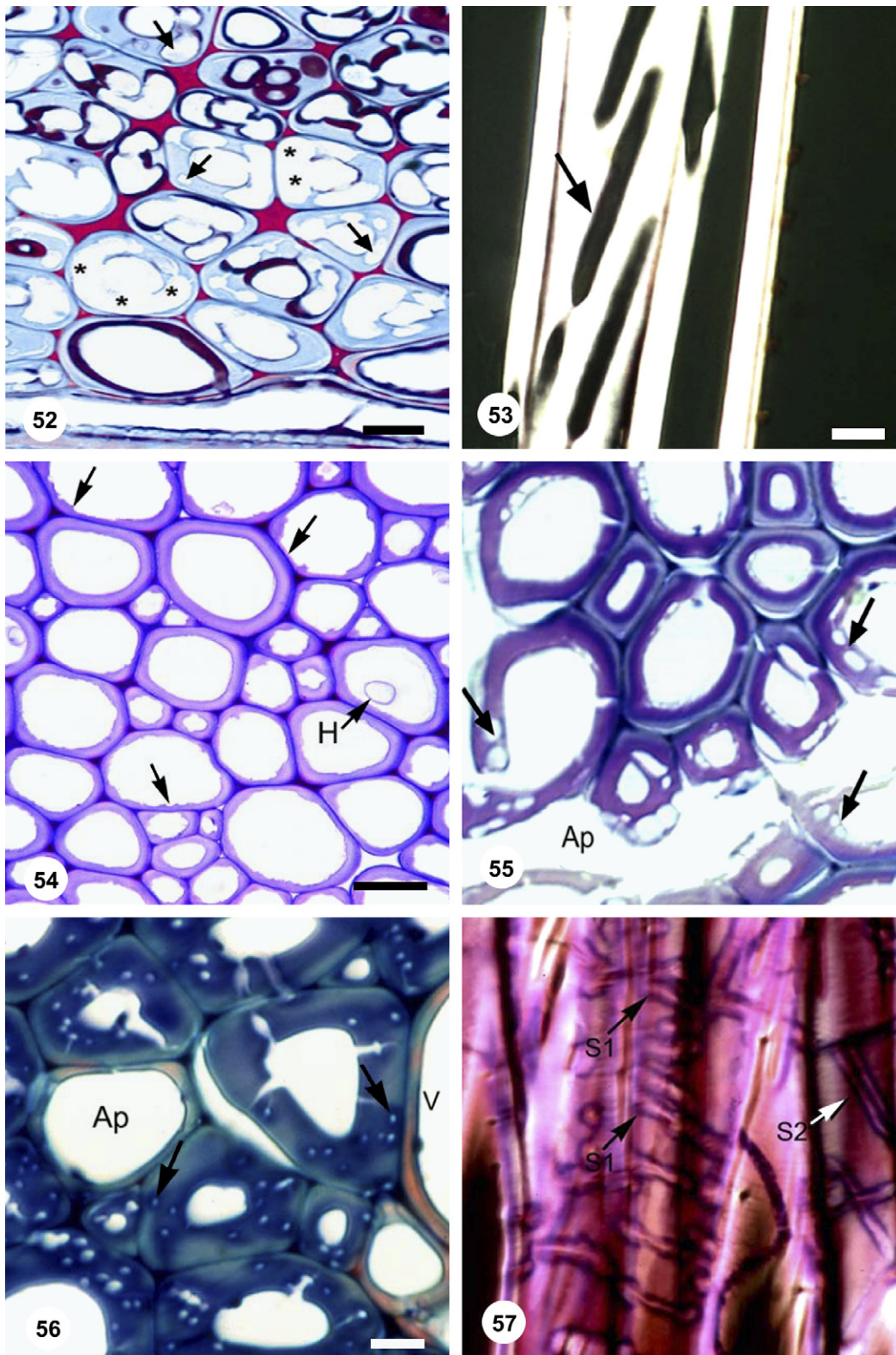
Soft rot caused by basidiomycetes

Cavity-forming soft rot decay is also caused by a range of basidiomycetes normally causing white- or brown rots (Table 3). The typical diamond-shaped or rhomboid cavities are formed in the wood cell walls following the helical course of cellulose microfibrils. Although early studies showed that some brown- and white rot fungi produce soft rot-like cavities within the S_2 layer (Duncan 1960; Liese 1961; Liese & Ammer 1964; Courtois 1965; Liese & Schmid 1966; Liese 1970; Nilsson & Daniel 1988) they were considered more likely to be associated with a local collapse of the cell wall, or an obscure white or brown rot degradation mode (Eriksson et al. 1990).

In beech and large-leaved lime wood *Meripilus giganteus* produced three types of cavities, which differed not only between hosts, but also between cell type and location within the annual ring (Schwarze & Fink 1998). Light microscopy showed that only one of the three modes of decomposition was reminiscent of a true soft rot and occurred within the naturally infected discoloured wood of beech. By contrast, cavity formation in artificially incubated wood of beech and large-leaved lime differed from a soft rot mode of attack, as extensive delignification always preceded cavity formation, and neither T-branching, L-bending, nor hyphal growth were found within cell walls. Moreover, the cavities were separated from one another within the secondary wall (S_2) by radial structures (Fig 55). The ability of *M. giganteus* hyphae within cell lumina to induce cavity formation in the adjacent walls is consistent with observations on a range of white rot fungi of diverse phylogenetic origin (Schwarze & Engels 1998). This degradation mode, which began with delignification, seems to occur under environmental constraints (moisture, oxygen, CO_2 etc) that are commonly encountered by root-decay fungi and wound parasites (Schwarze & Engels 1998). These conditions may also favour the diffusion of lignolytic enzymes into deeper regions of the cell wall (Eriksson et al. 1990).

The third type of cavity formation was exclusively associated with tension-wood fibres (Baum & Schwarze, 2001). Penetration of beech tension-wood fibres by perforation hyphae, and subsequent cavity formation, was associated with helical cracks i.e. incipient tension failures (Chow 1947). From large diameter hyphae, growing within the fibre lumen, numerous fine perforation hyphae extended transversely via helical cracks into the cell wall. Subsequent decomposition of cellulose within concentric layers resulted in the formation of 'half-moon' cavities (Nilsson & Daniel 1983) and rectangular boreholes in cell walls of tension wood fibres (Schwarze & Fink 1998).

A definite soft rot pattern has been seen in birch and Scots pine wood incubated with the white rot basidiomycete *Oudemansiella mucida* (Daniel et al. 1992). However, soft rot patterns have only recently been found in the wood of standing trees colonised by basidiomycetes. *Inonotus hispidus*, which decomposes heartwood and sapwood has been classified as a white rot fungus based on its lignolytic ability, although chemical



Figs. 52–57 – IX. Soft rot. (52) T.S. of beech wood naturally infected with *Kretzschmaria deusta* showing cavities (arrows) within the secondary walls of fibre-tracheids. Asterix, regions in which cavities have coalesced. Bar, 10 μm . (53) T.L.S. of Large-leaved lime wood artificially incubated with *Kretzschmaria deusta*. Chains of lenticular cavities (arrows) 10 – 60 μm long and with pointed ends (pointers) follow the orientation of the microfibrils. Bar, 10 μm . (54) T.S. of sycamore wood naturally infected with *Xylaria longipes* showing hyphal growth within the cell lumina of libriform wood fibres resulting in erosion of the secondary wall, i.e. soft-rot Type 2 (arrows). Bar, 20 μm . (55) T.S. of Large-leaved lime wood artificially incubated with *Meripilus giganteus*. Localized degradation of cell wall constituents results in the formation of cavities within the secondary walls

Table 3 – Range of wood degrading basidiomycetes reported to cause a soft rot Type 1

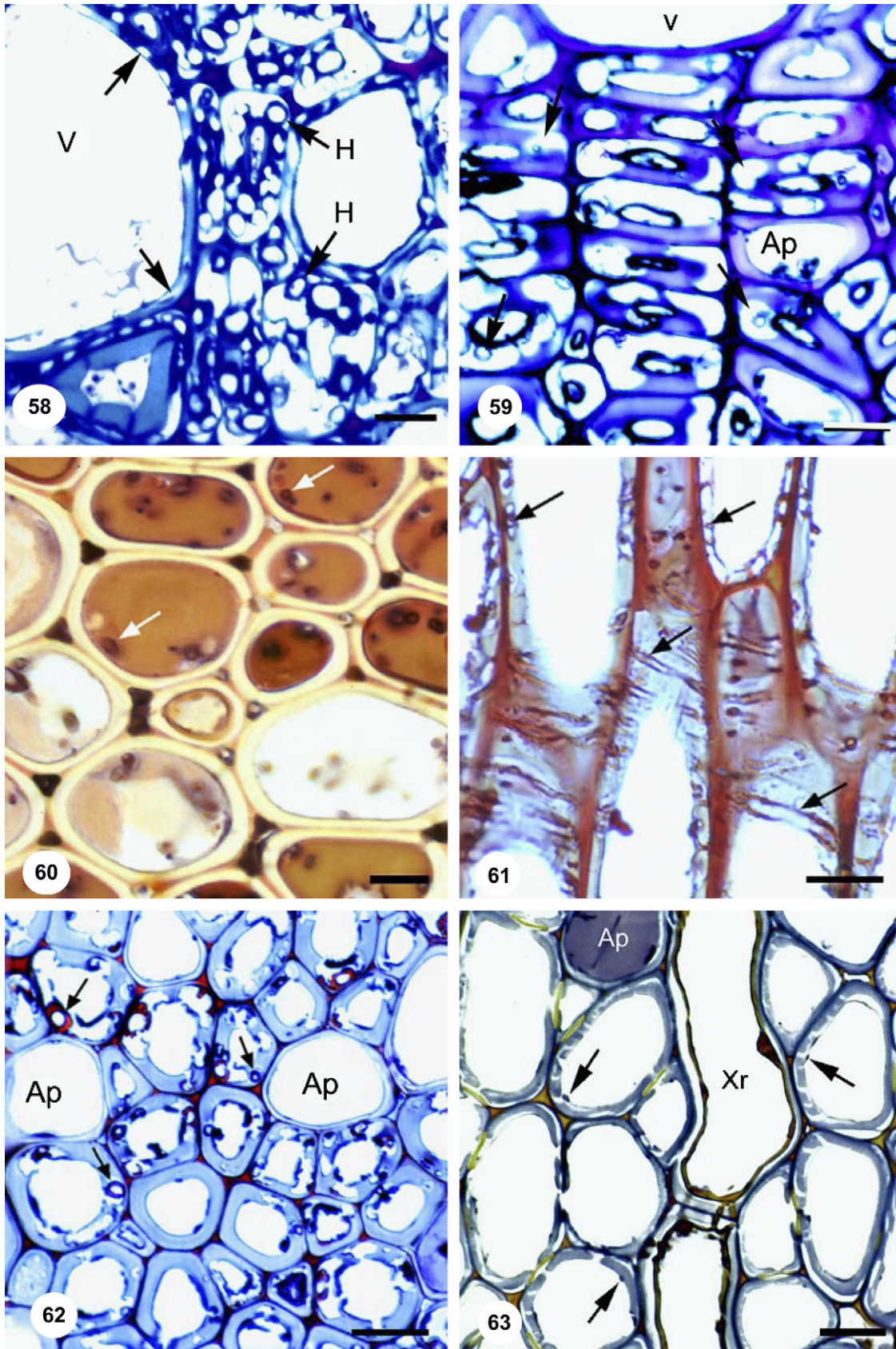
Wood decay fungus	Host	Conditions	Cell types	Authors
Brown rot fungi <i>Rigidoporus crocatus</i>	<i>Pinus nigrescens</i>	in vivo and in vitro		Duncan 1960; Courtois 1965
<i>Rigidoporus lineatus</i>	<i>Pinus eliottii</i> var. <i>eliottii</i>	fire salvaged logs stored under water sprinklers	tracheids	Hood et al. 1997
<i>Fistulina hepatica</i>	<i>Quercus robur</i>	in vitro and in the central heartwood of an approx 150 y old oak tree	libriform wood fibres, xylem ray parenchyma	Schwarze et al. 2000a
<i>Coniophora puteana</i>	<i>Entandrophragma cylindricum</i>	in vitro	libriform wood fibres	Kleist & Schmitt 2001
<i>Dacrymyces stillatus</i>	<i>Dipterocarpus</i> spp.	wooden handrail and in vitro	libriform wood fibres	Kleist et al. 2002
<i>Coniophora puteana</i>	<i>Pinus densiflora</i> , <i>Quercus acutissima</i>	in vitro	tracheidslibriform wood fibres	Lee et al. 2004
<i>Gleophyllum trabeum</i>	<i>Picea abies</i>	in vitro in THM-densified wood	tracheids, xylem ray parenchyma	Schwarze & Spycher 2005
White rot fungi <i>Armillaria borealis</i> , <i>Armillaria cepistipes</i> , <i>Armillaria gallica</i> , <i>Armillaria mellea</i>	<i>Picea abies</i> , <i>Fagus sylvatica</i>	roots of standing trees, in vitro	tracheids, fibre tracheids, vessels, xylem ray and axial parenchyma	Hartig 1878; Ferner 2004
<i>Heterobasidion annosum</i>	<i>Picea abies</i>	in vitro	tracheids	von Aufsess 1968
<i>Inonotus hispidus</i>	<i>Platanus x hispanica</i> , <i>Fraxinus excelsior</i>	in vitro and at the host-pathogen interface in standing trees	fibre tracheids, libriform wood fibres, xylem ray parenchyma	Schwarze et al. 1995; Schwarze & Baum 2000
<i>Oudemansiella mucida</i>	<i>Pinus sylvestris</i>	in vitro	tracheids	Daniel et al. 1992
<i>Meripilus giganteus</i>	<i>Fagus sylvatica</i>	in vitro and at the host-pathogen interface in standing trees	fibre tracheids	Schwarze & Fink 1998
<i>Auricularia auricularia</i>	<i>Betula pendula</i>	in vitro		Worrall et al. 1997
<i>Ganoderma adpersum</i>	<i>Fagus sylvatica</i>	in vitro and at the host-pathogen interface in standing beech trees	fibre tracheids	Schwarze & Ferner 2003
<i>Phellinus contiguus</i>	<i>Dipterocarpus</i> spp.	wooden handrail and in vitro	libriform wood fibres	Kleist et al. 2002

analysis has shown this to be rather low compared with many other basidiomycetes (Nutman 1929; Campbell 1931). It can cause a soft rot in addition or alternatively to its more typical mode of degradation in the cell walls of artificially incubated wood of London plane and of ash and in naturally infected standing trees of both hosts (Schwarze 1995; Schwarze et al. 1995a; Schwarze et al. 2004). The structure of the cavities and the formation of multiple L-bending by the associated hyphae were typical of a soft rot (Figs 56 and 57). Chains of minute cavities with biconical ends formed along the orientation of the cellulose microfibrils in the cell wall at early stages (Fig 57). They were joined by fine proboscis hyphae. In contrast to Type 1 soft rot caused by ascomycete and deuteromycetes, a lysis zone was formed around cavities by white rot basidiomycetes during their soft rot mode (Fig 56). This indicates that pre-delignification occurs before the actual cavity is formed

(Kleist et al. 2002). Depending on the fungal species and substrate colonized, a transition or switch mechanism is possible between white and soft rot. This phenomenon is encompassed in the term “facultative soft rot” as proposed by Schwarze et al (1995) for soft rot-like decay caused by basidiomycetes.

In the past, cavity formation was regarded as a distinct, relatively reliable character of soft rot, which can be used readily to differentiate this type of decay from other types of degradation (Zabel et al. 1985; Eriksson et al. 1990). However, the above studies strongly indicate that differentiation of decay types on the grounds of micro-morphological features, such as cavity formation and erosion troughs, is open to question. Conditions that seem to favour a soft rot mode of decomposition in standing trees by basidiomycetes appear to be related to the degree of lignification, lignin composition of cell walls

of libriform wood fibres. Single cavities are separated from each other by radial structures (arrows). Note absence of hyphae in the cell wall. Ap, axial parenchyma. Bar, 10 µm. (56) T.S. of London plane wood showing high frequency of cavities in secondary walls of fibre tracheids. Cell walls of axial parenchyma (Ap) and vessels (v) resist degradation. Note lysis zone in the cell wall around cavities. Note: Bar, 10µm. (57) R.L.S. of fibre tracheids of London plane wood and viewed under crossed Nicols, showing tunnel-shaped cavity formation along the S₁ and S₂ alignments of cellulose microfibrils. Bar, 10 µm. Figs 52–57 reproduced from Schwarze et al. (1999) by permission of the Rombach Verlag.



Figs. 58–63 – IX. X. Soft rot basidiomycetes. (58) T.S. of beech wood artificially colonized by *Armillaria cepistipes*. Hyphae (H) are apparent within cavities (arrows) of the secondary walls of vessels (v) and fibre tracheids. Bar, 10 μ m. (59) T.S. of beech wood artificially colonized by *Armillaria gallica*. Hyphae are apparent within secondary walls of fibres tracheids, whereas vessels (v)

and the presence of polyphenols, high moisture content or occluded cell lumina. Moreover, the ability of *I. hispidus* and other basidiomycetes to cause soft rot, either, in addition or alternatively to another mode of action, i.e. a white rot, may be a common phenomenon, which plays a significant role in colonization and lesion expansion in the xylem of standing trees.

Soft rot by *Armillaria* spp.

Armillaria sp. produced a Type 1 soft rot in Scots pine (Hartig 1878). Indeed light microscopy of beech wood submerged in agar and incubated with five *Armillaria* spp (*A. borealis*, *A. cepistipes*, *A. gallica*, *A. ostoyae* and *A. mellea*) revealed that after twelve weeks a soft rot mode of degradation was induced by most species (Ferner 2004). *Armillaria cepistipes* caused greatest soft rot Type 1 throughout a range of different cell types, including fibre tracheids, axial- and xylem ray parenchyma, and even vessels (Fig 58). In contrast, *A. mellea* exclusively caused selective delignification (Ferner 2004). *Armillaria ostoyae* caused a soft rot Type 1 in the secondary wall of fibre tracheids, but axial and xylem ray parenchyma showed no signs of soft rot. Moreover, the compound middle lamella resisted degradation even at advanced stages. *Armillaria gallica* caused both a soft rot and a simultaneous rot, but the latter was more prominent and soft rot Type 1 was only observed in close proximity to zone lines (Fig 59). Simultaneous rot was strongest in fibre tracheids regions between vessels, whereas fibre tracheids surrounding vessels and xylem ray parenchyma were resistant to decomposition (Fig 59). *A. borealis* caused weak Type 1 soft rot in the secondary wall of fibre tracheids within the latewood, along with simultaneous rot.

Dual modes of degradation by *Fistulina hepatica* in xylem cell walls of *Quercus robur*

Fistulina hepatica causes the condition known as Brown Oak (Cartwright 1937), in which the early stages of fungal development produce a reddish brown staining of the heartwood of oak (Schwarze et al. 2004) and sometimes sweet chestnut (*Castanea sativa*) (Groom 1915; Latham & Armstrong 1934; Cartwright 1937; Sachs et al. 1966; von Aufsess 1973; Krempf 1989). *F. hepatica* also causes the discolouration known as pencilling in jarrah (*Eucalyptus marginata*; Tamblyn 1937). After Brown Oak has been present for many years in a standing tree, it can progress to decay with cubical cracking, characteristic of brown rot (Cartwright & Findlay 1958; Wilcox 1978) but with limited effect on the mechanical properties of wood (Sachs et al. 1966; von Aufsess 1973; Krempf 1989). Previous

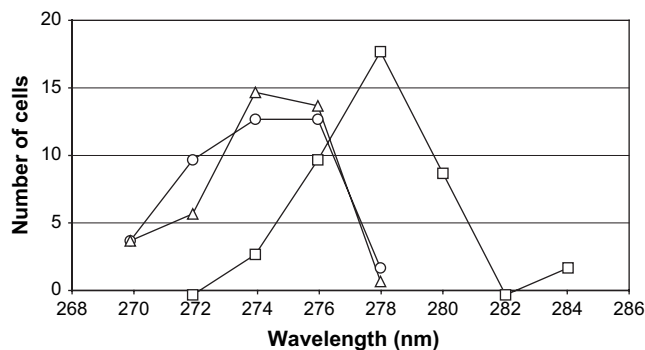


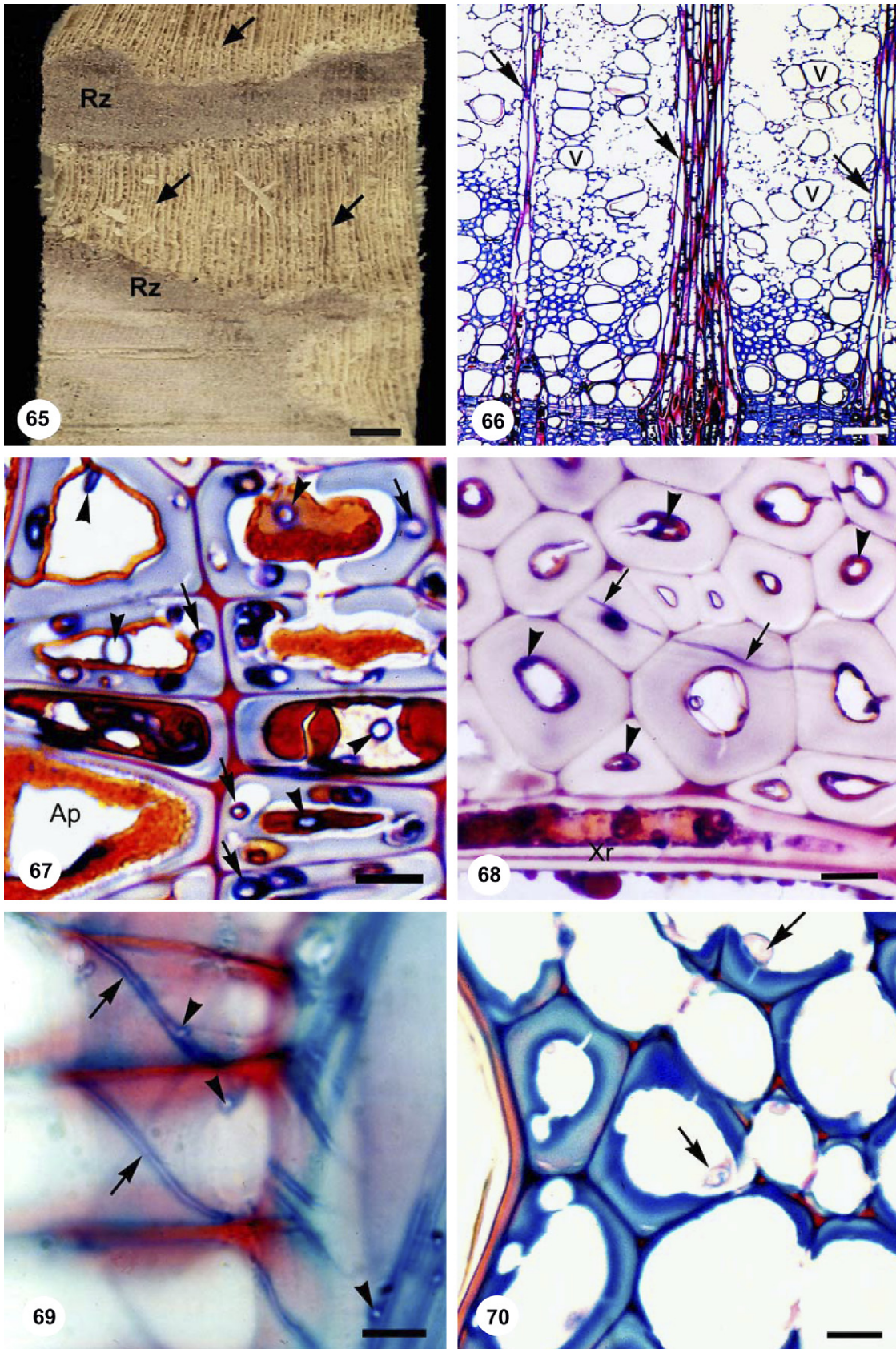
Fig. 64 – Soft rot basidiomycetes. UV-absorbance of secondary walls of oak. Number of cells showing a maximum at a given wavelength. Librifform wood fibres (O) and xylem ray parenchyma (Δ) show a different distribution from fibre tracheids (□). Figs 60–64 reproduced by Schwarze et al. (2000a) by permission of the British Mycological Society.

studies have failed to resolve the degradation mode of *F. hepatica* (Cartwright 1937; von Aufsess 1973). We have characterised the degradation mode of *F. hepatica* in naturally infected and artificially inoculated oak wood and determined why the fungus minimally affects strength properties (Schwarze et al. 2000a).

After 6 m, the deposition of brown materials within parenchyma cells containing hyphae was the only visible effect of colonisation (Fig 60). After 12 m, decomposition occurred but was initially confined to parenchyma cells of xylem rays in which the secondary walls showed helically orientated internal cavities containing hyphae, as in a soft rot (Fig 61). The hyphae were covered with a resinous material and persisted in a herringbone pattern after the secondary walls became heavily degraded (Fig 61). The adjacent librifform wood fibres also showed cavity formation (Fig 62). By contrast, fibre tracheids were mainly affected by a brown rot (Fig 63).

UV-microscopy indicated that cell types in which the soft rot mode occurred were rich in syringyl lignin, whereas the brown rot was associated with cells rich in guaiacyl lignin (Schwarze et al. 2000a) (Fig 64). Thus, *F. hepatica* possesses dual modes of degradation, which appear to correlate with the different lignin composition within cell types of oak (Yoshinaga et al. 1997; Schwarze et al. 2000a). In contrast to Type 1 soft rot caused by “white rot basidiomycetes”, there was no formation of a lysis zone around cavities, i.e. no pre-delignification. The degradation mode of *F. hepatica* may reflect the evolutionary status of different cell types in oak (Schwarze et al. 2000a). In this context, it is interesting to

and axial parenchyma (Ap) show no signs of degradation. Bar, 10 μm. (60) T.L.S. of oak wood naturally infected with *Fistulina hepatica*. Copious amounts of brownish hyphal secretions (arrows) fully occlude the lumina of xylem ray parenchyma cells. Ap, axial parenchyma. Bar, 25 μm. (61) T.S. of oak wood naturally infected with *F. hepatica*. Hyphae covered with resinous secretions (arrows) appear in a herringbone pattern within the secondary walls of xylem ray parenchyma cells. Bar, 10 μm. (62) T.S. of oak wood naturally infected with *F. hepatica*. Hyphae covered with resinous material (arrows) are apparent within the secondary walls of librifform wood fibres. Note absence of lysis zone in the cell wall around cavities. Bar, 10 μm. (63) T.S. of oak wood naturally infected with *F. hepatica*. Within the lumina of thin walled fibre tracheids, fine hyphae (arrows) are present growing on the S₃. Although the S₃ remains intact, minute cracks orientated perpendicular to the middle lamellae are apparent within secondary walls. Ap, axial parenchyma, Xr, xylem ray. Bar, 10 μm. Figures xx-xx reproduced from Schwarze et al. (2004) by permission of Rombach Verlag.



Figs. 65–70 – XI. Reaction zone penetration. (65) T.S. showing macroscopic appearance of London plane wood naturally decayed by *I. hispidus*. The presence of conspicuous multiseriate xylem rays (arrows) appearing as persistent ridges within the severely degraded wood is apparent. Note that although radial spread by *I. hispidus* is often demarcated by reaction zones

note that *F. hepatica* caused a brown rot in the relatively primitive fibre tracheids, but a soft rot in the more highly evolved libriform wood fibres. Some authors postulate that wood decay evolved slowly, beginning with the soft rot fungi from which white rot and subsequently brown rot fungi evolved (Worrall et al. 1997). The degradation modes of *F. hepatica* in oak may reflect a co-evolutionary adaptation to the different lignin compositions within more primitive and advanced cell types of oak.

As far as the effects of *F. hepatica* on wood strength in oak are concerned, it is significant that cell wall degradation occurs only after a prolonged period of colonisation. During this period, the fungus is probably able to obtain most of its nutrients from the phenolic contents of the cells in the heartwood. When decomposition begins, it involves the soft rot mode of the fungus, in which a tendency to brittle fracture develops, owing to the persistence of a lignin-rich skeleton. This skeleton consists of the most lignified components of the wood, which tend to preserve stiffness (Schwarze 1995). This probably explains why, during incipient stages of decay, impact-bending strength is reduced more than compression strength (Latham & Armstrong 1934; von Aufsess 1973; Krempel 1989).

Similar to *F. hepatica* a soft rot decay pattern was caused by the brown rot fungus *Coniophora puteana* in wood of Sapelli (*Entandrophragma cylindricum*; Kleist & Schmitt 2001). Soft rot occurred at high moisture contents. Interestingly, no lignin decomposition was caused by the fungus in close vicinity to the cavities, as demonstrated by UV-microspectroscopy. Lee et al. (2004) confirmed the latter results and demonstrated erosion and thinning of cell walls and decomposition of the lignin-rich middle lamellae, considered to be characteristic of white rot decay in *Pinus densiflora* and *Quercus acutissima* wood (Lee et al. 2004). Some strains of *C. puteana* had the capacity to cause a soft rot in tracheids of *P. densiflora* and libriform wood fibres of *Q. acutissima*.

8. Interactions at the host-fungus interface of the xylem

Decay in trees is a key consideration when assessing risks to people and property in areas of dense population or high

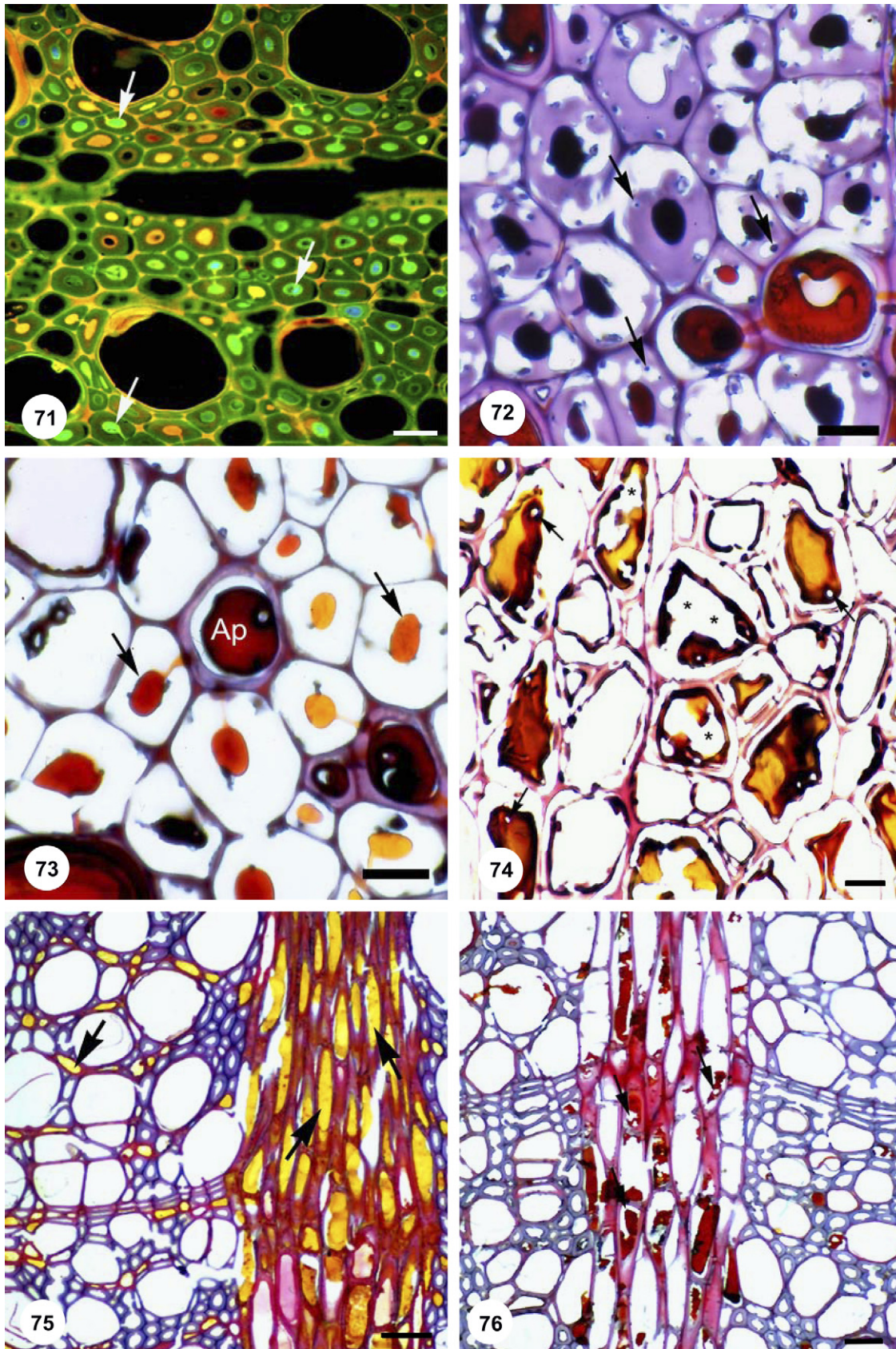
traffic volume, where major tree failures are likely to have serious consequences. Tree owners and managers are becoming increasingly aware of the need to undertake tree risk assessments, which include the accurate evaluation of decay and its effects on mechanical integrity (Lonsdale 1999).

Current concepts of the delimitation of fungal colonization in the xylem of trees are largely based on descriptions of patterns of discoloration and decay (Shain 1967, 1971, 1979; Shigo & Marx 1977; Smith 2006). Based on such observations, the CODIT-model (Compartmentalization of Decay in Trees) was developed and introduced by Shigo and Marx (1977). They proposed that there are distinct boundaries between a decay column and the surrounding sound wood, whereby the decay column is confined within a defined compartment. They recognized four different types of boundaries, corresponding to various anatomical interfaces within the xylem, and termed these "walls" 1, 2, 3, and 4. Walls 1 to 3 are formed in the wood extant at the time of wounding and represent anatomical features (Fig 78). Wall 4, the barrier zone, corresponds to pre-existing anatomical features, which may be modified by the deposition of materials laid down by the host as defensive barriers. Wall 4 also differs from walls 1 to 3 in that it is formed in the plane of the cambium in response to wounding (Fig 78).

Shain (1967, 1971, 1979) proposed the term reaction zone to describe the discoloured region between colonized and uncolonized xylem, which corresponds to walls 1 to 3 of the CODIT model, and distinguished it from the structurally distinct barrier zone. Another term, the column boundary layer, has more recently been applied to the altered tissue around a zone of colonized or dysfunctional xylem (Shortle & Smith 1990; Smith 2006). Unlike barrier zones, reaction zones are not structurally homogeneous and do not form a continuous morphological barrier to ingress of air and/or fungal attack (Pearce & Rutherford 1981; Boddy & Rayner 1983; Pearce 1990; Blanchette 1992; Fink 1999). In cases where a fungus grows beyond a reaction zone, it has been envisaged that the reaction zone migrates progressively into the previously functional xylem, being preceded by a margin of drying wood (the transition zone), and followed by incipient and eventually actual decay (Shain 1967, 1971, 1979).

More recently, there has been evidence that various fungal species are able to extend into functional sapwood some way beyond the reaction zone without stimulating the

(Rz) with a gummous appearance (arrows), these zones are readily breached by the fungus. Bar, 5 mm. (66) T.S. showing microscopic appearance of Fig. 65. The persistence of intact multiseriate xylem rays (arrows) as well as vessels (v) within the heavily degraded wood is apparent. This region was invaded by hyphae which had successfully breached the reaction zone. Bar, 120 µm. (67) T.S. of London plane wood naturally infected with *I. hispidus*. Abundant polyphenolic deposits are apparent within the lumen of axial parenchyma (Ap) and adjacent fibre tracheids. Degradation of cell walls occurs only where the hyphae have escaped adverse conditions by growing in the cell wall (arrows). In contrast, hyphae within the lumina (pointers) are arrested and have no visible effect on the cell wall structure. Bar, 10 µm. (68) T.S. showing hyphal growth in the reaction zone of London plane. Within regions of the reaction zone, hyphal growth (arrows) is confined to the secondary walls of fibre tracheids. Hyphal growth occurs rarely within the lumina of cell elements which are blocked with polyphenolic deposits (pointers). Xr, xylem ray. Bar, 10 µm. (69) R.L.S. of London plane wood, showing bore holes (pointers) and tunnel-shaped cavities (arrows) of indefinite length in the secondary wall of a multiseriate xylem rays. Hyphae are visible in the tunnels. Bar, 10 µm. (70) T.S. showing hyphal growth in wood beyond the reaction zone of London plane. The more advanced zones of decay in the naturally infected wood showed typical white rot degradation, with erosion of secondary walls by hyphae (arrows) growing on the S₃ layer. Cavities are only occasionally evident. V, vessel. Bar, 10 µm. Figs. 65–70 reproduced from Schwarze et al. (1999) by permission of the Rombach Verlag.



Figs. 71-76 - XII Host-fungus-interactions. (71) T.S. of reaction zone in beech wood stained with acridine orange and viewed under the fluorescence microscope. Polyphenols within the lumina of fibre tracheids appear green-brown. Bar, 30 μ m. (72) T.S. of a reaction zone in beech wood artificially incubated with *Kretzschmaria deusta* showing soft rot and dark hyphae

simultaneous migration of the zone. Instead, a new reaction zone forms later at the new colonization front. This type of discontinuous host response may be recognized by the presence of reaction zone relics within decayed wood (Fig 65) (Pearce 1991; Schwarze & Fink 1997). In such interactions, the reaction zone essentially acts as a static boundary to decay, and not as the product of a dynamic host response.

Mechanisms of reaction zone penetration by decay fungi in the xylem standing trees

The mechanisms by which decay fungi may overcome host barriers have so far been the subject of only few studies. Light microscopy studies revealed that the white rot fungus *Inonotus hispidus* was able to penetrate reaction zones in London plane by tunnelling through the cell walls like a soft rot fungus, thus circumventing phenolic deposits within the cell lumina (Figs 67 and 68; Schwarze and Fink (1997). The interaction between London plane and *I. hispidus* resulted in a very conspicuous degradation pattern, characterised by the presence of breached reaction zones and prolonged persistence of multi-serial xylem rays even at advanced stage of decomposition (Figs 65 and 66). The pattern is suggestive of a counteraction between the invasiveness of the fungus and boundary-sealing responses of the tree. The invasiveness of *I. hispidus* may be due to its ability to circumvent inhibition in the xylem, caused by accumulated polyphenolic deposits, which is accomplished by its ability to grow within the cell wall (Figs 67–69). One important aspect of the soft rot decay mechanism is that once fungal hyphae have penetrated into the cell wall and branched along the orientation of the cellulose microfibrils, they are able to circumvent toxic compounds that may be present in the cell lumen (Figs 67–69). This can apply to wood preservative chemicals impregnated into the wood, but equally to natural products and extractives deposited in the lumina within reaction zones (Schwarze & Fink 1997; Schwarze & Baum 2000; Schwarze et al. 2004). Within these zones, tyloses or gummy deposits block the lumina of vessels and fibres adjacent to parenchyma. In many cases, toxic phenolic compounds are present, so that hyphal growth is both physically and chemically deterred within the cell lumina, which would otherwise provide easy pathways for fungal colonisation and surfaces for cell wall erosion (Schwarze & Fink 1997). Once a reaction zone has been breached, it is possible for the living sapwood to form a new reaction zone. However, this may not readily happen during periods of tree dormancy. Thus, there may be

periods when the host is not able to respond to invasion by *I. hispidus*, so that the fungus can revert to a white rot mode with hyphal activity in the cell lumina (Fig 70).

Mechanisms of reaction zone penetration by decay fungi in wood of beech and large-leaved lime

As explained by Shigo and Marx (1977), the structure of woody tissues contains boundaries, beyond which microbial spread is initially deterred. The boundaries divide functional from dysfunctional wood, and the extent of the dysfunctional zone is determined by the nature of the wound or other traumata that exposed the wood to the atmosphere. The water potential of the wood at that time also partly determines the extent of dysfunctional zone, since it influences the initial extent of the ingress of air into the wood (Boddy & Rayner 1983; Boddy 1992). The boundaries are often reinforced in sapwood by active defences, which form dark coloured reaction zones (Fig 71), but there is often no way of knowing whether a decay fungus will eventually grow beyond such a zone, or how rapidly it might do so. These questions are often important in hazard tree assessment, especially in cases where a tree is not immediately deemed to be hazardous, but could become so in the future. The effectiveness of reaction zones in restricting fungal growth is largely dependent on factors within the tree. Some fungal species may, however, be better adapted than others to breach these boundaries. This is apparent from the example of *I. hispidus* in London plane.

Beech and large-leaved lime wood blocks containing naturally induced reaction zones and challenged by the three basidiomycetes *Inonotus hispidus*, *Ganoderma adspersum*, and *Fomitopsis pinicola*, and one ascomycete, *Kretzschmaria deusta* (Schwarze & Baum 2000; Baum & Schwarze 2002). All the fungi, except *F. pinicola*, breached reaction zones, but the mechanisms involved were all somewhat different. Both *I. hispidus* and *K. deusta* bypassed blocked cell lumina in beech by tunnelling through cell walls (soft rot mode), but the latter caused far more decomposition of cell walls. Decomposition of polyphenols was slight with *I. hispidus* and absent with *K. deusta* (Figs 72 and 73). By contrast, *G. adspersum* preferentially degraded the polyphenolic occlusions in the cell lumina. The failure of *F. pinicola* to invade reaction zones was typical of a brown rot fungus having limited enzymatic potential and a uniform growth pattern. In contrast to beech, the cell lumina in the reaction zone of large-leaved lime contained only sparse deposits, which were easily degraded by all fungi and

(arrows) within cavities of fibre tracheids. Note polyphenolic deposits within the cell lumina of fibre tracheids and axial parenchyma. Bar, 10 μm . (73) T.S. of a reaction zone in beech wood artificially incubated with *Kretzschmaria deusta* showing the persistence of brownish polyphenolic deposits (arrows) and compound middle lamellae in the otherwise strongly degraded wood; polyphenols derived from an axial parenchyma cell (Ap) Bar, 10 μm . Figs. 71–73 reproduced from Schwarze and Baum (2000) by permission of Blackwell Publishing. (74) T.S. of a reaction zone in large-leaved lime wood naturally infected with *Kretzschmaria deusta* showing soft rot and dark hyphae (arrows) degrading polyphenols within libriform-wood fibres. Asterix, heavily degraded polyphenols. Axial parenchyma, Ap. Bar, 10 μm . Reproduced from Baum and Schwarze (2002) by permission of Blackwell Publishing. (75) T.S. of a reaction zone in London plane wood showing tyloses (pointers) and accumulation of polyphenols (arrows) in lumina axial and xylem ray parenchyma. Bar, 50 μm . Reproduced from Schwarze and Ferner (2003) by permission of AB Academic Publishers. (76) T.S. of a reaction zone in London plane wood naturally infected with *Ganoderma adspersum*. Most polyphenols within parenchyma cells have been degraded (arrows) by the fungus. Bar, 50 μm . Reproduced from Schwarze and Ferner (2003) by permission of AB Academic Publishers.

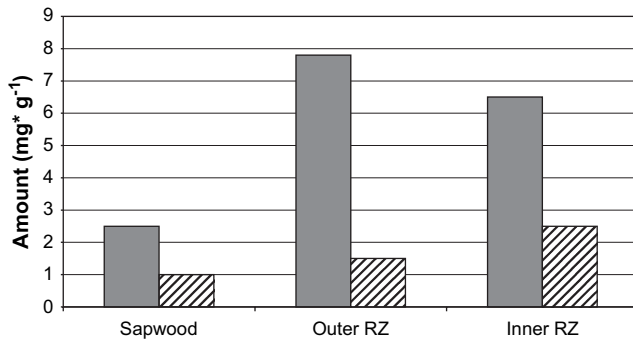


Fig. 77 – Reaction zone penetration. Cell-wall bound amounts of proanthocyanidins in beech (black columns) and large-leaved lime (hatched columns) within the sapwood, outer and inner reaction zone (RZ), as measured by spectrophotometric analysis at a wavelength of 550 nm. Reproduced from Baum and Schwarze (2002) by permission of Blackwell Publishing.

did not prevent the passage of hyphae between adjacent cells via pits when reaction zones within excised wood blocks were challenged *in vitro* with the decay fungi (Fig 74). In a comparison of extracts from the reaction zone of large-leaved lime and the stronger reaction zone of beech, differences were found in the concentrations (Fig 77) and composition of induced antimicrobial compounds (Baum & Schwarze 2002). Thus, the reaction zone of large-leaved lime was relatively weak, compared with the static reaction zone of beech, and migrated

into the functional sapwood at an active microbial invasion front as a result of a dynamic interactive process.

Invasiveness of *Ganoderma* species in wood containing naturally induced reaction zones

Among amenity and roadside trees, the presence of *Ganoderma* spp. is often taken to indicate that a hazard assessment may be necessary. Identification to species-level is frequently not attempted, despite the availability of information about the hazard-potential of particular species (Schwarze & Ferner 2003). The relative abilities of *Ganoderma applanatum*, *G. resinaceum* and *G. adspersum* to overcome the reaction zone of London plane were assessed under controlled conditions using wood blocks containing naturally induced reaction zones (Fig 75). Also, the effect of physically damaging the reaction zone on fungal spread was examined (Fig 76). During incubation, fungal entry into each block was controlled by coating it with paraffin wax, so as to leave only one side available for colonisation (Schwarze & Ferner 2003).

There were clear differences between the fungi, on the basis of both histological criteria and drilling resistance measurements, after four and eight week's incubation (Schwarze & Ferner 2003). Both *G. adspersum* and *G. resinaceum* breached the reaction zone, but *G. adspersum* was more aggressive in this respect (Schwarze & Ferner 2003). *Ganoderma adspersum* became well established in the xylem ray parenchyma within the reaction zone, preferentially degrading polyphenols but not the cell walls. *Ganoderma resinaceum* occupied an intermediate position amongst the three fungal species studied. Like

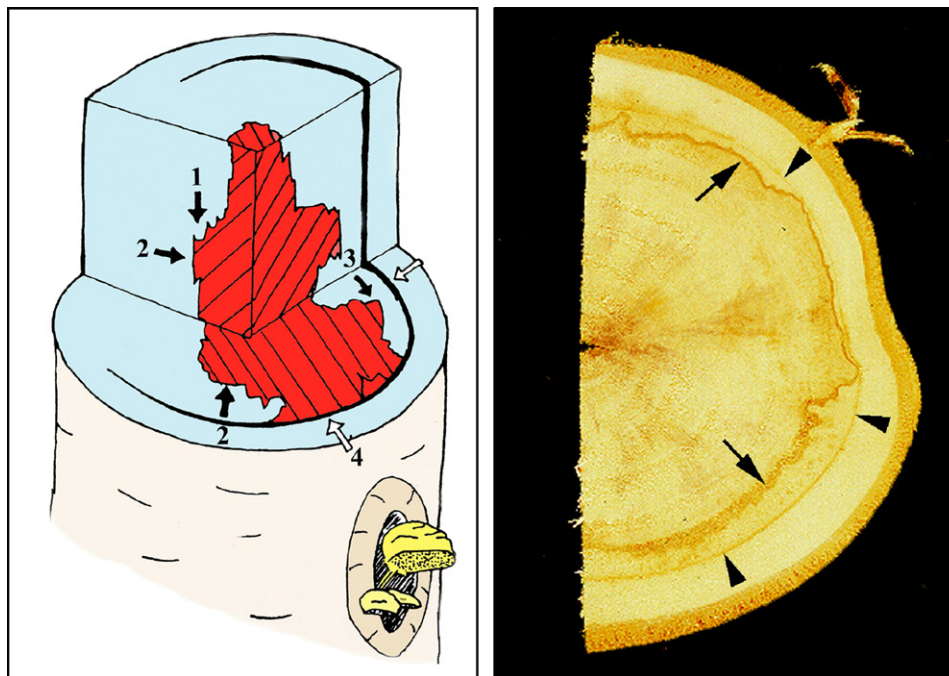


Fig. 78 – Reaction zone penetration. Left: Schematic representation of walls 1–4 of the CODIT Model. The most effective of the walls is ‘wall 4’ (equated in the model with the barrier zone), which is formed after injury of the cambium. Right: Cross-section of lime stem, containing a barrier zone (arrow heads), which sharply demarcates the decayed and discoloured wood from the sound sapwood. The wood formed before the initiating injury contains a reaction zone (arrows), but there are many kinds of decay fungi that can breach reaction zones in lime trees (Schwarze et al. 2004).

G. adspersum, it modified the polyphenolic deposits in the reaction zone, but without degrading them to the same extent. On the other hand, *G. applanatum* did not breach the reaction zone, due to an inability to modify the defensive compounds in this region. When wood blocks were drilled before incubation, so as to breach the reaction zones physically, *G. applanatum* was able to colonise and degrade the adjacent sapwood. This pre-treatment also enhanced the ability of *G. resinaceum* and *G. adspersum* to enter the sapwood. *Ganoderma resinaceum* preferentially exploited the sapwood before starting to degrade the polyphenolic barrier of the reaction zone, whereas *G. adspersum* began to degrade this barrier at an early stage. Within wood, containing no reaction zones, *G. applanatum* caused the most rapid loss of cell wall structure, followed by *G. resinaceum* and *G. adspersum* (Schwarze & Ferner 2003).

The ability of *G. adspersum* to penetrate intact reaction zones could clearly be a hazard factor, by allowing the development of potentially extensive decay, even within trees of high vitality. Trees colonised by this species may often have adequate residual walls of sound wood at the time of initial assessment. However, they seem to have a poor long-term prognosis, compared to trees colonised by *G. applanatum*, which probably remains confined within previously dysfunctional columns of wood (Schwarze & Ferner 2003). *Ganoderma resinaceum* occupies an intermediate position in this respect, which could mean that, despite having some ability to penetrate reaction zones in the wood of *Platanus*, it can be kept in check within trees of high vitality. Although both *G. applanatum* and *G. resinaceum* are less likely than *G. adspersum* to cause very extensive decay, they can both cause an intense decomposition of the wood that they occupy. The major differences between these *Ganoderma* species make it essential to identify them correctly, for hazard risk assessment.

In summary our studies of wood decay under the microscopes show that the boundaries between different types of fungal decay are less clear cut and there is a much greater diversity in the way different decay fungi challenge their hosts and substrates. Evidence has been provided that the terms brown rot, white rot and soft rot may not be obsolete, but rigid definitions for fungi that are placed into these categories may be less appropriate than thought previously (Eaton 2000).

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