Bamboo inhabiting fungi and their damage to the substrate

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To: University of Hamburg, Ph.D. Dissertation Committee for Dongsheng Wei

As a native speaker of the English language, I have checked and made minor editorial corrections to the Ph.D. dissertation entitled, **Bamboo inhabiting fungi and their damage to the substrate**, authored by Dongsheng Wei. I find that this dissertation is very well written and fulfills the requirements for an English dissertation.

Respectively,

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Abstract

As a fast growing and sustainable grass, bamboo is of increasing interest for construction and industrial utilization. However, bamboo has a low natural durability and is easily attacked by fungi during storage, transport, processing and final use. Little is known about the fungi inhabiting and degrading the bamboo. Therefore, fungal identification and degradation studies were performed with bamboo species.

Fungi were isolated from culms of bamboo species and identified by rDNA-ITS sequencing. In total, 150 strains were isolated and 76 isolates were identified representing 16 genera and 37 species. Most isolates were Deuteromycetes/Ascomycetes (moulds, stain fungi). Two Basidiomycetes, *Schizophyllum commune* and *Cyathus stercoreus*, were identified. Genera of *Aspergillus* and *Penicillium* contain closely related species. Therefore, ß-tubulin and calmodulin sequences were also used for identification.

Staining experiments were performed with pure cultures of blue-stain fungi in Petri dishes. Colonization of vessels and parenchyma, observed by light microscopy after 4 and 20 weeks incubation, occurred by thick, brown hyphae, chlamydospores and penetration of lignified cell walls by transpressoria as it is also found in wood cells.

The degradation of bamboos by white-, brown- and soft-rot fungi was investigated in preserving jars, Kolle flasks, vermiculite, and in the field.

In preserving jars, the mass loss (ML) was measured with small samples (3 × 1 cm) after 1, 3 and 12 months of incubation with Basidiomycetes for white-rot and brown-rot decay and with Ascomycetes for soft-rot attack. Whereas the brown-rot fungi *Coniophora puteana* and *Gloeophyllum trabeum* yielded less degradation, the white-rot fungus *Trametes versicolor* and the soft-rot species *Chaetomium globosum* revealed considerable decay. ML was significantly affected by the water content of bamboos: *C. puteana* was aggressive in dry samples (approx. 30 % moisture content), the white-rot species *Schizophyllum commune* produced most ML in wet substrates (180 %u).

To imitate natural conditions, bigger samples (25 cm long) were used in the "Fungus cellar-test" where the bamboo is inoculated with pure cultures and incubated in large metal tubes containing unsterile garden soil. In contrast to results with small samples in the jar test, samples in the Fungus cellar were considerably more degraded by *C. puteana* (max. 43 % ML) and *S. commune* (max. 20 %). Obviously, the moisture conditions influenced both fungi, whereby *S. commune* decayed samples with soil contact and thus with high water content (90-182 %u) more than samples without soil contact; in contrast, *C. puteana* produced more ML in samples placed on wood supports and with lower water content (34-65 %u).

The durability of five bamboo species from various origins against brown-, white- and soft-rot fungi was investigated in Kolle flasks in accordance to the European standards EN 350-1, EN 350-2 and EN 113. Considerable variability exists in the durability of the bamboos: *Guadua angustifolia* was rather resistant to *T. versicolor* and *Dendrocalamus asper* against *Ch. globosum*. Among the brown-rot fungi, four strains of *C. puteana* and two strains of *G. trabeum* produced less ML (max. 2.9 %). Of the white-rot fungi, *T. versicolor* yielded the highest ML (max. 15.3 %), whereas *S. commune* was rather inactive (max. 3.2 %). Of the soft-rot fungi, *Ch. globosum* showed medium degradation (max. 9.6 %) and *Paecilomyces variotii* low decay (max. 3.1 %).

Proposed is a method to investigate degradation of lignocelluloses by pure cultures of basidiomycetes using preserving jars with vermiculite as reservoir for water and nutrients. Samples of *Gigantochloa atroviolacea* and *Phyllostachys pubescens* and wood samples of *Fagus sylvatica* and *Pinus sylvestris* were inoculated with *C. puteana* and *S. commune*. The fungi were cultured on vermiculite containing different amounts of tap water or malt extract solution. ML of the bamboos after 32 weeks was low and did not show a remarkable influence of moisture content and nutrient addition. However, considerable degradation of *P. sylvestris* sapwood occurred by *C. puteana* whereby moisture and nutrients influenced its aggressiveness.

Bamboo stake test was conducted in the field. Fungal activity was visually assessed after 3.5 years. The opened stakes showed different stagess of degradation.

Chemical analyses were performed with hydrolysis and HPAEC-borate. *Pleurotus ostreatus* and *T. versicolor* consumed the three cell wall components hemicellulose, cellulose and lignin. The brown rot fungi *C. puteana* and *G. trabeum* were less aggressive. The soft-rot fungus *Ch. globosum* consumed the carbohydrates, however with some decrease in lignin content, which is characteristic of this rot type.

The micromorphology of *Bambusa maculata* degraded by the white-rot fungi *Cyathus stercoreus, P. ostreatus, T. versicolor* and the soft-rot fungus *Ch. globosum* was studied by transmission electron microscopy (TEM) and ultraviolet microspectrophotometry (UMSP). TEM results showed morphological changes in the various cell wall layers. The UMSP area scans and additional point analyses provided insights into the topochemistry of decay.

In summary, the results show that all bamboo species investigated can be colonized by the various groups of fungi, moulds, staining and rot fungi. Considerable degradation occurs by white- and soft-rot fungi.

Zusammenfassung

Bambus ist als schnellwüchsiges, nachwachsendes Gras zunehmend interessant für Konstruktionen und industrielle Nutzung. Wegen der geringen natürlichen Dauerhaftigkeit wird Bambus jedoch während Lagerung, Transport, Verarbeitung und Nutzung leicht von Pilzen angegriffen. Da über Besiedlung und Abbau wenig bekannt ist, erfolgten Pilzidentifizierungen und Abbauversuche an mehreren Bambusarten.

Von Bambushalmen aus verschiedenen Ländern wurden insgesamt 150 Pilzstämme isoliert und 76 durch rDNS-ITS-Sequenzierung identifiziert, die zu 16 Gattungen und 37 Arten gehören. Meist handelte es sich um Deuteromyceten bzw. Ascomyceten (Schimmelpilze und Bläue-Erreger) sowie um die Basidiomyceten *Schizophyllum commune* und *Cyathus stercoreus*. Da die Gattungen *Aspergillus* und *Penicillium* nahe verwandte Arten enthalten, wurden zusätzlich die ß-Tubulin- und Calmodulin-Sequenzen zur Identifizierung mittels BLAST verwendet.

In Petrischalen erfolgten Verfärbungsversuche an Bambus mit Bläuepilz-Reinkulturen. Die Lichtmikroskopie von Gefäßen und Parenchymzellen nach 4 und 20 Wochen Inkubation zeigte die für Bläuepilze in Holzgewebe typischen dicken, braunen Hyphen und Chlamydosporen sowie ihr Durchdringen lignifizierter Zellwände mit Transpressorien.

Der Abbau von Bambus wurde mit Weiß-, Braun- und Moderfäulepilzen in Weckgläsern, Kolleschalen, auf Vermiculit und im Freiland untersucht.

In den Gläsern wurde der Masseverlust (MV) an kleinen Proben (3 x 1 cm) nach 1, 3 und 12 Monaten Kultivierung von Basidiomyceten hinsichtlich Weiß- und Braunfäule sowie Ascomyceten auf Moderfäule gemessen. Während die Braunfäulepilze *Coniophora puteana* und *Gloeophyllum trabeum* nur wenig Abbau bewirkten, ergaben der Weißfäulepilz *Trametes versicolor* und der Moderfäuleerreger *Chaetomium globosum* beträchtliche Zersetzung. Die Bambusfeuchte beeinflusste den MV deutlich: *C. puteana* war in trockenen Proben (etwa 30 % Wassergehalt) aktiver und der Weißfäulepilz *Schizophyllum commune* in nassem Substrat (180 %u).

Zur Nachahmung natürlicher Bedingungen wurden größere Proben (25 cm lang) im „Pilzkeller“ auf unsteriler Gartenerde in großen Metallwannen mit Reinkulturen inkubiert. Im Gegensatz zu den kleinen Abmessungen im Weckglasversuch zeigten hier *C. puteana* (maximal 43 % MV) und *S. commune* (max. 20 %) deutlichen Abbau. Offensichtlich wurden beide Pilze durch die Feuchtebedingungen beeinflusst: *S. commune* zersetzte Bambus mit Erdbodenkontakt und daher höherem Wassergehalt (90-182 %u) stärker als ohne

Bodenberührung, wogegen *C. puteana* mehr MV bei den auf Unterlagen befindlichen Proben und somit geringerer Feuchte (34-65 %u) bewirkte.

An fünf Bambusarten verschiedener Herkunft wurde die Dauerhaftigkeit gegenüber Weiß-, Braun- und Moderfäulepilzen in Kolleschalen gemäß der Europäischen Standards EN 350-1, EN 350-2 und EN 113 ermittelt. Die Arten unterschieden sich beträchtlich: *Guadua angustifolia* war recht dauerhaft gegenüber *T. versicolor* und *Dendrocalamus asper* gegen *Ch. globosum*. Innerhalb der Braunfäuleerreger ergaben die vier Stämme von *C. puteana* und die zwei Isolate von *G. trabeum* wenig Abbau (max. 2,9 % MV). Von den Weißfäulepilzen bewirkte *T. versicolor* den größten MV (max. 15,3 %), während *S. commune* weniger aktiv war (max. 3,2 %). Bei den Moderfäuleerregern zeigten *Ch. globosum* mittleren (max. 9,6 %) und *Paecilomyces variotii* kaum Abbau (max. 3,1 %).

Zur Untersuchung des Lignocellulose-Abbaues mit Basidiomyceten-Reinkulturen wurde eine neue Kultivierungsmethode mit Vermiculit als Reservoir für Wasser und Nährstoffe vorgeschlagen. Proben von *Gigantochloa atroviolacea* und *Phyllostachys pubescens* sowie Holzproben von *Fagus sylvatica* und *Pinus sylvestris* wurden mit *C. puteana* und *S. commune* beimpft und auf Vermiculit mit verschiedenem Gehalt an Leitungswasser oder Malzextrakt-Lösung inkubiert. Bei Bambus war der Abbau nach 32 Wochen zwar gering und ohne erkennbaren Einfluss von Wasser- und Nährstoffzugabe, erheblich jedoch bei *P. sylvestris*-Splintholz und *C. puteana*, dies in Abhängigkeit von Bambusfeuchte und Nährstoffen.

Die visuelle Auswertung eines Freilandversuches (stake test) nach 3,5 Jahren ergab unterschiedliche Zersetzungsstadien sowie meist Moderfäule in der äußeren Bambusschicht und Weißfäule im Inneren.

Laut chemischer Analysen nach Hydrolyse und HPAEC-Borat-Technik veratmeten *Pleurotus ostreatus* und *T. versicolor* die Zellwandbestandteile Hemicellulose, Cellulose und Lignin, *C. puteana* und *G. trabeum* waren kaum aktiv, *Ch. globosum* verbrauchte Fäuletyp-charakteristisch besonders beide Kohlenhydrate und minderte den Ligningehalt.

Die Mikromorphologie wurde an *Bambusa maculata* nach Abbau durch *Cyathus stercoreus*, *P. ostreatus*, *T. versicolor* und *Ch. globosum* mittels Transmissions-Elektronenmikroskopie (TEM) und Ultraviolett-Mikrospektralphotometrie (UMSP) untersucht. Die TEM zeigte morphologische Veränderungen der Zellwandschichten, der UMSP-Flächen-Scan und die Punktmessungen ergaben Einblicke in die Topochemie.

Insgesamt haben die Ergebnisse gezeigt, dass alle untersuchten Bambusarten von den verschiedenen Pilzgruppen, Schimmelpilze, Bläue-Erreger und Fäulnispilze, besiedelt und durch Weiß- und Moderfäulepilze stark angegriffen werden.

List of abbreviations

BLAST	Basic local alignment search tool
CLSM	Confocal laser scanning microscopy
CML	Compound middle lamella region
CREA	Creatine agar
CYA	Czapek yeast extract agar
FTIR	Fourier transform infrared
HPAEC	High performance anion exchange chromatography
HPLC	High performance liquid chromatography
ITS	Internal transcribed spacer
MC	Moisture content
MEA	Malt extract agar
ML	Mass loss
ML-F	Middle lamella of fibre
ML-P	Middle lamella of parenchyma cell
PCR	Polymerase chain reaction
RH	Relative humidity
SEM	Scanning electron microscopy
TEM	Transmission electron microscope
UMSP	Ultraviolet microspectrophotometry
UV	Ultraviolet
XPS	X-ray photoelectron spectroscopy
YES	Yeast extract sucrose agar

1 Introduction

1.1 Fungi

Approximately 120,000 fungal species have been described, and new species are added at the rate of approximately 1,200 per year (Blackwell 2011, Kirk et al. 2008). A conservative estimate of the total number of fungal species thought to exist is 1.5 million (Hawksworth 1991, 2001). Blackwell (2011) even estimated over 5 million fungal species.

If 1.5 million fungal species is a reasonable estimate, the vast majority of all extant fungi are yet to be named. Assuming a relatively constant rate at which new species are described, it will take more than 1,100 years to catalogue and describe all remaining fungi. However, many of these fungi are likely to become extinct before they are ever discovered given current rates of habitat and host loss. For example, up to 2% of the tropical forests are destroyed globally each year (Purvis and Hector 2000). These habitats are exceedingly rich in fungal species (Hawksworth and Rossman 1997). For example, 15-25 % of the fungi collected in short-term studies in the tropics are new species (Kirk et al. 2008).

1.1.1 Characterization

The following descriptions are mainly based on Kendrick (1992), Alexopoulos et al. (1996), Jennings and Lysek (1999) and Schmidt (2006). A fungus is a member of a large group of eukaryotic organisms with cells devoid of chlorophyll that includes microorganisms such as yeasts and moulds as well as the more familiar mushrooms. These organisms are classified as a kingdom: Fungi, which is separate from plants, animals, protists and bacteria. Some morphological, biochemical, and genetic features are shared with other organisms, while others are unique to fungi, clearly separating them from the other kingdoms. One major difference is that fungal cells have walls that contain chitin, unlike the cell walls of plants and some protists, which contain cellulose, and unlike the cell walls of bacteria. These and other differences show that fungi form a single group of related organisms, named the Eumycota (true fungi or Eumycetes), that share a common ancestor. This fungal group is distinct from the structurally similar Myxomycetes (slime moulds) and Oomycetes (water moulds).

Fungi mostly develop from very narrow, tubular, branching filamentous structure called hyphae, which are the main mode of vegetative growth. A mass of hyphae collectively forms a spreading network called mycelium. These filaments exude enzymes, and absorb food at their growing hyphal tips. Moreover, hyphae are collectively very long so that they can explore and exploit food substrates very efficiently. Reproduction of fungi, usually by means of spores by which develop directly on substrates, are released by the hyphae or by a range of unique and complex structures formed on the fungal mycelium that are called fruiting bodies.

Fungi play an essential role in the decomposition of organic matter and nutrient recycling. They have long been used as a direct source of food, such as mushrooms and truffles, as a leavening agent for bread, and in fermentation of various food products, such as wine, beer, and soy sauce. Since the 1940s, fungi have been used for the production of antibiotics, and, more recently, various enzymes produced by fungi are used industrially and in detergents. Fungi are also used as biological antagonists to control weeds, plant diseases and insect pests. Many species produce bioactive compounds called mycotoxins, such as alkaloids and polyketides, which are toxic to animals including humans. The fruiting structures of a few species contain psychotropic compounds and are consumed recreationally or in traditional spiritual ceremonies. Fungi can break down manufactured materials and buildings, and become significant pathogens of humans and other animals. Losses of crops due to fungal diseases (e.g. rice blast disease) or food spoilage can have a large impact on human food supplies and local economies.

1.1.2 Classification

In the classification of fungi, there have benn various attempts that focused on artificial and natural systems. However, a generally recognized fungal classification system does not exist, which was ironically argued that there might be as many systems as there are systematists.

The differences between fungi and other organisms regarded as plants had long been recognized. Whittaker (1969) proposed a five-kingdom system and recognized fungi as an additional kingdom. It has become a commom standard; some refinement of this standard is still used in the literature and forms the basis for new multi-kingdom systems. He classified the polyphyletic origin of the fungi into Protista and Fungi. Protista with about 2,000 described species, are grouped into sex divisions that include slim fungi and ‘lower fungi’,

which are independent from each other. The ‘higher fungi’, with about 120,000 described species, group into four division: Zygomycota, Ascomycota, Basidiomycota and Deuteromycota (Schmidt 2006).

Due to recent research technology based on DNA comparisons, the taxonomy of the fungi is in a state of constant flux. The molecular revolution in fungal taxonomy commenced in the early 1990s, with analyses of PCR-amplified ribosomal RNA genes (White et al. 1990). These current phylogenetic analyses often overturn classifications based on older and sometimes less discriminative methods based on morphological features and biological species concepts obtained from experimental matings.

As the broad outlines of fungal phylogeny have come into focus, there have been repeated attempts to summarize the state of knowledge and to restructure higher-level classifications. Two important works that have influenced fungal taxonomy in the 21st century are Ainsworth & Bisby’s Dictionary of the Fungi (Kirk et al. 2001) and The Mycota VII (McLaughlin et al. 2001a, b). These publications represented major advances toward a phylogenetic classification of Fungi, but they have become outdated.

There is consequently a pressing need for the fungal systematics community to adopt by consensus, a higher-level classification for Fungi that is based on well-supported monophyletic groups, and which can be recommended for general use. Hibbett et al. (2007) presented a higher-level classification for all groups of Fungi according to recent molecular phylogenetic studies. Currently, one kingdom, one subkingdom, seven phyla, ten subphyla, 35 classes, 12 subclasses, and 129 orders are accepted. The seven phyla include Microsporidia, Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, Glomeromycota, Ascomycota, and Basidiomycota. The subkingdom Sikarya contained the Ascomycota and the Basidiomycota.

As there is no unique accepted system at the higher taxonomic levels, there are frequent name changes at every level. Researchers are encouraged to use a unified and more consistent nomenclature. The Amsterdam Declaration on Fungal Nomenclature (Hawksworth et al. 2011) was agreed at an international symposium convened in Amsterdam on 19-20 April 2011 under the auspices of the International Commission on the Taxonomy of Fungi (ICTF). The declaration recognizes the need for an orderly transitition to a single-name nomenclatural

system for all fungi and to provide mechanisms to protect names that otherwise become endangered. Websites such as Index Fungorum and ITIS list current names of fungal species, with cross-references to older synonyms.

1.1.3 Identification

1.1.3.1 Traditional methods

Ever since the pioneering 18th and 19th century taxonomical works of Carl Linnaeus, Christian Hendrik Persoon, and Elias Magnus Fries, fungi have been classified according to their morphology (e.g., characteristics such as spore colour or microscopic features) or physiology. The first records of fungi on bamboo are *Dothidea goudotii* from the leaves of *Chusquea* sp. and *Sphaeria bambusae* from the culms of *Bambusa arundinacea* (Leveille 1845).

To identify the fungi, fruit bodies are preferentially used (Grosser 1985, Breitenbach and Kränzlin 1986, Jahn 1990, Ryvarden and Gilbertson 1993, 1994, Bravery et al. 2003, Schmidt 2006, Huckfeldt and Schmidt 2006). However, fruiting bodies are not always present. Fruiting body formation after isolation in laboratory culture is rare, e.g. in *Antrodia vaillantii* and *Schizophyllum commune*. However, some tree parasites like *Armillaria* species form rhizomorphs that can be used for identification. Several indoor species form mycelial strands (cords) which allow identification by the classical strand diagnosis of Falck (1912) and the extended version of Huckfeldt and Schmidt (2006). Even for experts, it may be difficult to recognize closely related species. Morphological characteristics between two related species are rather difficult to distinguish from each other. Furthermore, morphological characteristics within a species are quite variable so that two individuals may not necessarily be recognized as belonging to the same species.

Three Basidiomycetes with their different fruiting bodies and mycelia on malt extract agar plates are shown in Fig. 1.1.

Fig. 1.1. Example of Basidiomycetes with characteristic fruiting bodies but similar mycelia. Left: *Pleurotus ostreatus*; middle: *Schizophyllum commune*; right: *Trametes versicolor*.

1.1.3.2 Modern methods

Classical methods could be rapid if there are fruiting bodies or further characteristics. If only vegetative mycelia are seen, (e.g., on living trees, on stored and fallen timber, in buildings or in laboratory culture), keys and books for identification are available (Nobles 1965, Stalpers 1978, Lombard and Chamuris 1990). However, some genera among fungi can barely be distinguished at the species level in culture, as is true for the indoor genera *Antrodia*, *Coniophora* and *Leucogyrophana*. Thus, molecular methods, like protein electrophoresis, isozyme analyses, immunological and DNA-based techniques, were established in the 1980s (Schmidt 2006) and are used for identification.

Nowadays, the fungal ribosomal DNA (rDNA) most often used for fungal identification. Particularly, specific DNA-fragments from the cluster of ribosomal RNA genes, the ITS sequence between the 28S and 18S rDNA within a species are highly conserved but differ even between closely related species. Sequencing these can allow an unambiguous identification, irrespective of fungal classification. To amplify the internal transcribed spacer (ITS) regions, Kim et al. (2011) identified fungi from bamboo isolates in Korea using ITS5

and ITS4 as primers for ascomycetes and ITS5 and ITS4B as primers for basidiomycetes. ITS4 and ITS1 as primers were applied for identifying strain and mould fungi on bamboo in China (Ma and Jiang 2009).

1.1.4 Important genera of bamboo inhabiting fungi

More than 1,100 species of fungi have been described or recorded from bamboo, which comprise about 630 Ascomycetes, 150 Basidiomycetes and 330 mitosporic taxa (100 Coelomycetes, and 230 Hyphomycetes) (Hyde et al. 2002). Most species belong to the Ascomycetes, Basidiomycetes or Deuteromycetes (moulds), but had been only identified by classical methods, thus may contain false identifications.

In the following notes a description of common and relevant fungi is given which were identified in this study by DNA techniques. For a more detailed account of the specific species, see references provided in this section.

Aspergillus

Among the moulds, *Aspergillus* species are common contaminants on bamboo. They occur more normally than *Penicillium* spp in subtropical and tropical areas. It is important to identify aspergilli that many species are human pathogens (e.g. *A. fumigatus*, *A. terreus*) or notorious mycotoxin producers (e.g. *A. flavus*, *A. versicolor*). Some species can produce useful nature products that can be used into industry (e.g. *A. niger*).

To all *Aspergillus* species, *aspergillum* is still the name of an asexual spore-forming structure; although one-third of species are known to have a sexual stage. The Colonies of *Aspergillus* usually fast growing, coloured white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores. Conidiophores are unbranched with a vesicle. Phialides are grown directly on the vesicle (uniseriate) or on metulae (biseriate). Conidia are one-celled, smooth or ornamented, and hyaline or pigmented, forming chains which maybe compact columns (columnar) or diverging (radiate). Species may produce `Hülle´ cells (large, thick- and smooth-walled cells) or sclerotia (firm, usually globose, masses of hyphae). Teleomorphs consist of *Eurotium*, *Emericella*, *Neosartorya* and other genera.

Morphological characteristics were mainly used in the identification of *Aspergillus*. The genus is divided into 18 groups and 132 species are recongnized (Raper and Fennell 1965). Up to now, more than 250 species with about 70 named teleomorphs are identified. Modern molecular technology is also used in the identification (Samson and Varga 2007, Varga and Samson 2008).

Cladosporium

The genus of *Cladosporium* is widely distributed in the world. Some species are pathogens or are saprophytic and more or less host-specific on old or dead bamboo.

The colonies of *Cladosporium* are usually slow growing, coloured olive-green to blackish-brown or black. Conidiophores are fragile, erect, straight or flexuous, unbranched or branched only in the apical region, with geniculate sympodial elongation in some species. Conidia are one- or 4-celled, ellipsoidal, fusiform, ovoid or (sub) globose, verrucose or echinulate, often with distinct scars, pale to dark olivaceous-brown, smooth-walled, which are produced in branched acropetal chains.

In the past decades, only a few indoor species are recognized and identified as *C. cladosporioides, C. sphaerospermum*, *C. herbarum* and *C. macrocarpum*. The latest phylogenetic studies demonstrated that many new species may be discovered from these taxa. (Schubert et al. 2007, Zalar et al. 2007, Dugan et al. 2008).

Penicillium

The genus of *Penicillium* is composed of over 400 species. Many species are growth on various substrates. Furthermore, some species are found on bamboo where they spoiled and coloured the bamboo and bamboo products. *Penicillium chrysogenum*, *P. brevicompactum* and *P. citrinum* are the most common ones.

The typical mycelium consists of hyphae, which is a highly branched network, septate, and usually colourless. The conidiospores sprout on the mycelia, which are the main dispersal organ of the fungi and usually green. Sexual reproduction produces ascospores.The asci contain eight unicellular ascospores.

The morphological characteristics can be used for classification, but the isolates should be culture under standard conditions. However, the phenotypic characteristics of some species are very similar so that it is very difficulty to distinguish. The latest identification of *Penicillium* is based on a polyphasic approach, using a combination of the morphology of the conidiophores and conidia, colony characteristics on various media and temperatures and molecular data (Frisvad and Samson 2004, Samson and Frisvad 2004). An electronic key for the identification of *Penicillium* subgenus *Penicillium* species is provided at http://www.cbs.knaw.nl/penicillium/, which is based on a database using phenotypical and molecular (ß-tubulin sequences) characteristics. A database for identifying indoor penicillia is also obtainable at http://www.cbs.knaw.nl/indoor/ (Adan and Samson 2011).

Schizophyllum

Within the basidiomycetes, *Schizophyllum commune* is a worldwide distributed fungus, occurring on every continent except Antarctica. It causes white-rot decay of wood and also bamboo, acting as a ubiquitous saprophyte and opportunistic pathogen. The basidioma has a shell-shaped cap, with the tissue concentrated at the point of attachment, resembling a stem. It is often wavy and lobed, with a rigid margin when old. It is tough, felty and hairy, and slippery when moist. It is greyish white and up to 4 cm in diameter. The gills producing basidiospores on their surface are pale reddish or grey, very narrow with a longitudinal split edge, split when the fruiting body dries out and become inrolled when again wet, which is unique among the fungi. It is found predominantly from autumn to spring on dead wood or living plant tissue, in coniferous and deciduous forests. Fig. 1.2 shows fruiting bodies on a bamboo culm in Nigeria.

Fig. 1.2. Fruiting bodies of *Schizophyllum commune* on bamboo (photo: W. Liese).

1.2 Bamboo

Bamboo is one of the most important non-timber forest products among the world's plant and forest resources. It is a tribe of flowering perennial evergreen plants and belong to the Monocotyledons, which are grouped in the kingdom Plantae, order Poales, family Poaceae, and subfamily Bambusoideae. At present, three tribes are recognized, which are supported by molecular phylogenetic results, and reflect the three main lineages of Bambusoideae (Sungkaew et al. 2009): Arundinarieae (temperate woody bamboos, 28 genera, 533 species), Bambuseae (tropical woody bamboos, 66 genera, 784 species) and Olyreae (herbaceous bamboos, 21 genera, 122 species). Altogether, 1,439 species in 115 genera of bamboo are being described and reported worldwide (Clark 2012), which are mainly distributed in the tropical and sub-tropical zone and a few in the temperate and frigid zone. Bamboo usually grows with tree species in mixed forests below the main canopy. According to their geographical distribution, they are divided into three regions: the Asian-Pacific, the American and the African region (Jiang 2007) (Fig. 1.3). In the 1920s, bamboo was introduced to Europe, North America and Australia; thus, new cultivating bamboo regions appeared that are different from their natural distribution regions.

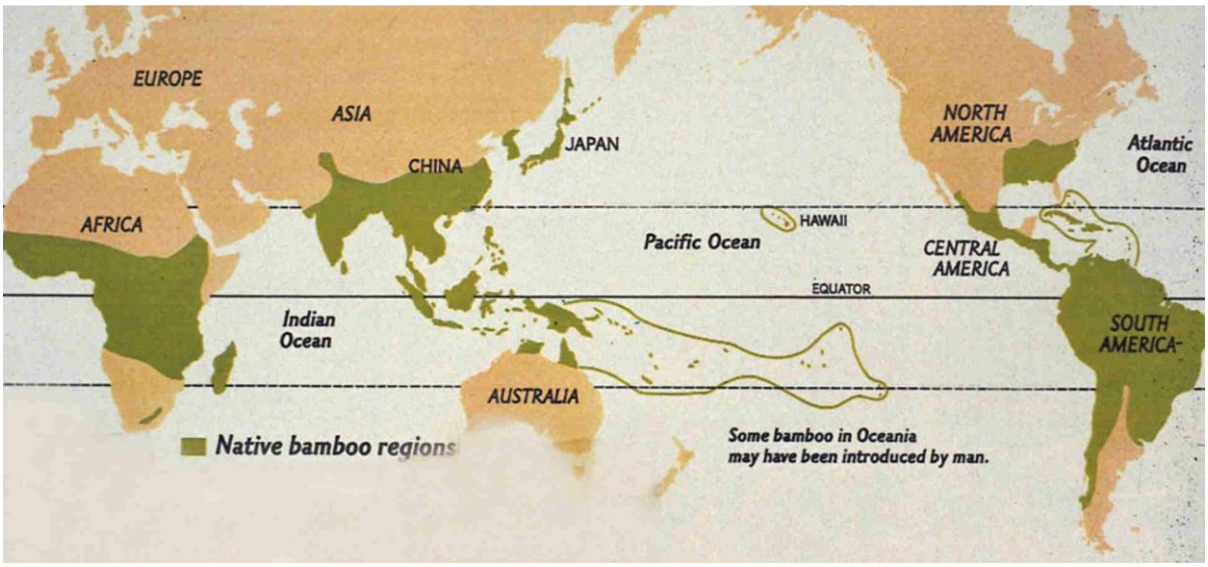

Fig. 1.3. Geographic distribution of bamboo (from W. Liese).

In the past, bamboo did not receive much utilization, but since the start of the 20th century, it quickly began to appear as a secondary bamboo forest in tropical and sub-tropical forests due to its fast growth and strong propagation after the upper canopy species were cut. Because of its diverse usage and the high ecological and economic value, bamboo was planted at a large

scale and the artificial bamboo forest was developed (Fig. 1.4). The secondary and artificial bamboo forest is expanding by the strong underground rhizomes. The total forest area on this land decreased year by year. According to the statistics in the past 50 years, the cover rate of the world forest declined from 25 to 17%, the tropical forest cover is disappearing by 240,000 thousand km^2 every year and 0.46 km^2 every minute (Zhou 1999). On the contrary, the area of bamboo forests increasingly expands, at a steady annual rate of 3% (Sun and Duan 2004).

Fig. 1.4. Bamboo (*Phyllostachys pubescens*) forest in China (photo: W. Liese).

1.2.1 Biology

1.2.1.1 Morphology

The main structure of the bamboos consists of three parts: a root system, a subterranean rhizome system and the aerial culm system.

Bmboo growth originates from a unique rhizome system. There are two different growth patterns (Fig. 1.5): sympodial species, in which the rhizome remains at the clump, such as *Dendrocalamus gigantea*; and monopodial species (`running bamboos'), which spread with their rhizome widely underground and send up new culms, such as *Phyllostachys pubescens.*

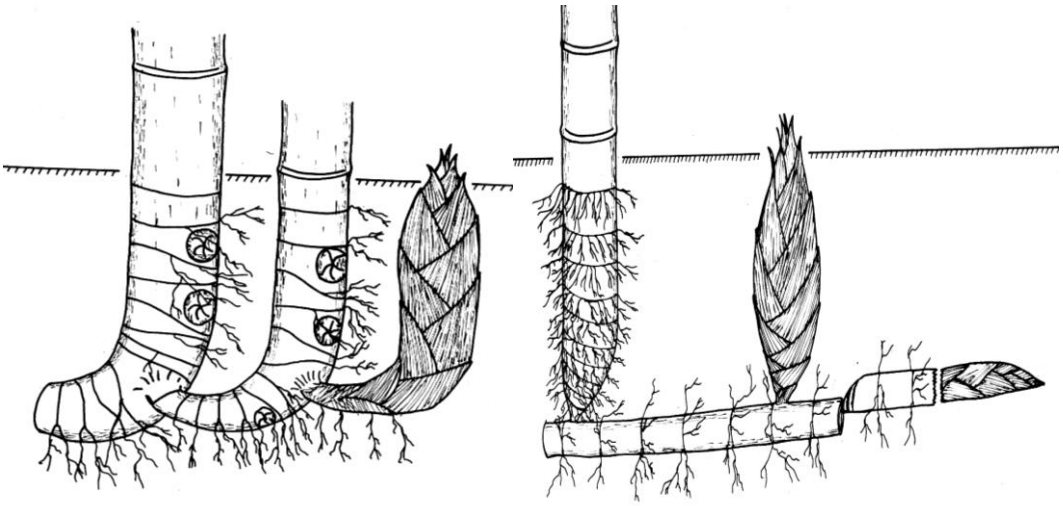

Fig. 1.5. Sympodial (left) and monopodial (right) bamboo (from W. Liese).

Unlike trees, there is no secondary growth of the culm. The culm emerges from the rhizome at its full diameter. The typical bamboo culms are composed of nodes and internodes. It is mostly hollow and separated at the nodes. It grows to its full height in one growing season. Its maturation takes 3 to 5 years according to different species. Due to lack of a cambium, bamboo does not possess the ability to enlarge the diameter of the culm. In some species, the culm can grows to several metres in height; in contrast, some species just grow to a few centimetres high.

Most bamboo species flower infrequently, at intervals of 30 – 80 years, exhibiting `mass flowering ´with all plants of a particular species flowering worldwide over a period of a few years. After flowering, they die (Fig. 1.6).

Fig. 1.6. Bamboo flowering.
Left: *Fargesia murielae*; right: *Dendrocalamus strictus* (photos: W. Liese).

1.2.2.2 Anatomy of culm

The culm is the most important part of bamboo and the main resource for utilization. Due to rare flowering, classification of bamboo is mainly based on the culm structure. The physical and mechanical properties of bamboo wood closely correlate to its anatomical structure. From outer to inner on the cross section, the basic structure of a culm consists of an epidermis, cortex, ground tissue and lacuna.

The epidermis is the outermost layer of the culm, containing elongated cells, a short cork and silica cells, and the stomata. The outer cell wall usually is covered by tiny protuberances, cutinized layer and wax coating. Size, shape, distribution and arranging of protuberances vary in different bamboo species. Most of them diffuse in aggregates, and the others are reticulate, grid-shape or undulating. Some species have unicellular hairs on the epidermis. The characteristics of the culm surface can be considered for taxonomy.

Under the epidermis lies the hypodermis, which consists of 2-3 layers of thick-walled sclerenchymatous cells. But the hypodermic cell of pachymorph bamboo has thin walls and is undistinguishable from the cortex. The thickness of cell wall varies depending on its location. Epidermis and hypodermis are usually called `bamboo green´, because the cells contain chlorophyll.

The cortex lies between hypodermis and ground tissue, which consists of several layers of parenchyma cells.

The ground tissue consists of parenchyma cells embedding the vascular system. The parenchyma tissue has two types of vertically positioned cells: the long and short parenchyma cells. The long parenchyma cells, with thickened polylamellated and lignified wall, usually have starch granules in them. The short parenchyma cells are scattered among the long parenchyma cells and characterized by dense cytoplasm, thin walls and no lignification. On average, the culm consists of about 52 % parenchyma, 40 % fibres and 8 % conducting tissue, but these values vary with species. The cell types vary considerably within a culm, both transversally across the culm wall and vertically along the culm. The conducting tissue increases slightly from the outer part in the middle third of the culm wall. The percentage of

parenchyma in the inner third is considerably higher than in the outer third. Correspondingly, the percentage of fibres decreases from the outside to the inside.

Vascular bundles are embedded in the parenchyma tissue. The phloem is on the outside of the vascular bundle, and the xylem is on the inside. The vascular bundles of the bamboo internodes are composed of two metaxylem vessels, phloem, protoxylem and attached fibre sheaths. Some species have additional fibre bundles. The appearance of vascular bundles across the culm varies continuously from the periphery towards the centre. The bundles in the middle of the culm wall usually exhibit a maximum size and a characteristic form.

The classical work about vascular bundles types was performed by Grosser and Liese (1971) analyzing 52 species in 14 genera. They investigated the variability of vascular bundles in form and size and grouped them into four basic types (Fig. 1.7). In 1984, Wen et al. added the "semiopen type". The five basic types have often been used in further studies.

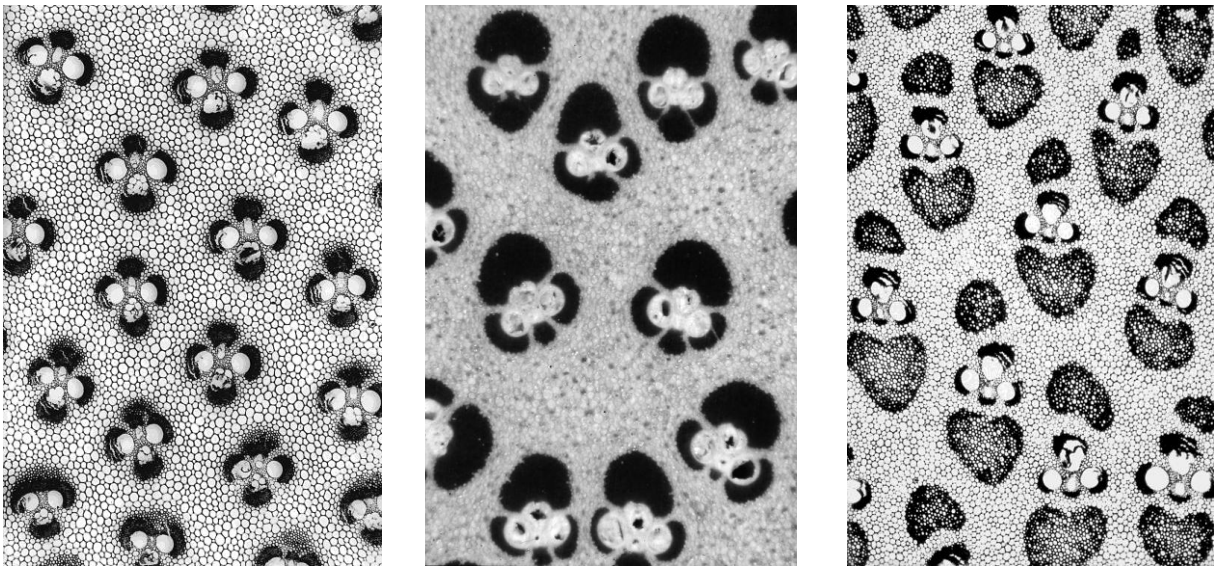

Fig. 1.7. Basic types of vascular bundles.
Left: type 1 with fibre sheaths; middle: type 2 with enlarged fibre sheath with the phloem; right: type 3 with two isolated fibre bundles (photos: W. Liese).

1.2.2.3 Chemical composition of the bamboo culm

The main constituents of the culm tissue are cellulose, hemicellulose and lignin; minor constituents consist of various other soluble polysaccharides, protein materials, resins, tannins,

waxes and a small amount of ashes. The main chemical constituents of bamboo are not too different from those of woody materials (Vena et al. 2013).

Cellulose is the fundamental material of the bamboo cell wall. Cellulose is a macromolecular polysaccharide. Wood cellulose consists of up to 15,000 ß-1,4 linked glucose anhydride units. The content of cellulose in bamboo of 40-60% is higher than that in hardwood. Vena et al. (2013) summarized that for bamboo, glucan content was 40-53 %, whereas the glucan content of softwoods and hardwoods is typically 40–52 % and 38-56 %, respectively. The content in 14 bamboo species located in the Yunnan Province in China was found to be 48.3 % on average (Wang et al. 1999).

More than 90 % of bamboo hemicellulose is xylan. Hardwood xylan, like that of *Fagus sylvatica*, consists of about 200 ß-1,4 linked xylose units with side chains of arabinose and 4-*O*-methyl-D-glucuronic acid (Schmidt 2006). The main hemicelluloses of bamboo are 4-*O*-acetyl-4-*O*-methyl-D-glucuronoxylans, which account for approximately 25 % of the cell wall material. Bamboo xylan contains 6-7 % acetyl groups, whereas hardwood xylan has 8-17 % (Vena et al. 2013). The chemical analyses in Chapter 3.5 also revealed that bamboo xylan is mainly composed of xylose, arabinose, galactose and 4-*O*-methyl-D-glucuronic acid.

Lignin is a macromolecular three-dimensional polymer. Bamboo lignin belongs to the grasses (monocotyledons) type of lignin consisting of three types of phenyl-propane units: *p*-hydroxylphenyl (H), guaiacyl (G) and syringyl (S) units interconnected through biosynthetic pathways in a molar ratio of 10:68:22 (Jiang 2007). This shows that bamboo lignin is qualitatively, but not quantitatively, similar to hardwood lignin.

The distribution of chemical constituents of bamboo cell walls differs and varies in the same bamboo with age and cell differentiation stages.

1.2.2 Significance

Growing attention of the global markets to bamboo commodities is a relatively new phenomenon. Developing countries today are not interested in the development at any price. They are increasingly interested in sustainable economic, social and environmental

development. Bamboo is an excellent resource for promoting sustainable growth, international trade and South-South and South-North cooperation.

The recent development of bamboo is often called a "golden revolution," analogous to that of the "green revolution," which resolved some world food security problems in the 1970-1980s. The ongoing golden revolution may help continue to resolve global wood security problems and reduce environmental pressure on forests. Growing international trade of bamboo products is an indication of the growing importance of bamboo for the developing and the developed nations.

1.2.3 Uses

Bamboo has high economic and cultural significance, being used as food source, construction material, and for versatile products.

Bamboo has traditionally been used to make a wide range of everyday utensils, particularly in Japan, where archaeological excavations have uncovered bamboo baskets dating to the late Jomon period (2000-1000 BC). Bamboo has a long history of use in Asian furniture. For example, Chinese bamboo furniture has a distinct style based on a millennia-long tradition.

Bamboo, like true wood, is a natural composite material with a high strength-to-weight ratio useful for structures. In its natural form, bamboo as a construction material is traditionally associated with the cultures of South Asia, East Asia and the South Pacific, to some extent in Central and South America, and by extension in the aesthetic of the Tiki culture. Bamboo is used for houses (Fig. 1.8) and structures (Fig. 1.9). In China and India, bamboo is used to hold up simple suspension bridges, either by making cables of split bamboo or twisting whole culms of sufficiently pliable bamboo together. Bamboo has also long been used as scaffolding; the practice has been banned in China for buildings over six storeys, but is still in continuous use for skyscrapers in Hong Kong. In the Philippines, the nipa hut is a fairly typical example of the most basic sort of housing where bamboo is used; the walls are split and woven bamboo, and bamboo slats and poles may be used as its support. In Japanese architecture, bamboo is used primarily as a supplemental and/or decorative element in buildings such as fencing, fountains, grates and gutters, largely due to the ready abundance of quality timber.

Fig. 1.8. Bamboo for houses in Ethiopia, Bali, China and Germany (from top left to bottom right) (photos: W. Liese).

Fig. 1.9. Bamboo for structures (photos: W. Liese).

Bamboo based panels and floorings (Fig. 1.10) are made through a series of mechanical and/or chemical processes, including addition of adhesives. They are pressed at a certain temperature and pressure, and featured in large standard sizes, with good and stable physical and mechanical properties: There is a big potential for bamboo based panels and floorings to be used as engineering materials for decorative and/or structural purposes.

Fig. 1.10. Bamboo for furniture and flooring (photos: W. Liese).

Bamboo fibres have been used to make paper in China for centuries. A high-quality, handmade paper is still produced in small quantities. Coarse bamboo paper is still used to make spirit money in many Chinese communities.

Bamboo pulps are mainly produced in China, Myanmar, Thailand and India, and are used for printing and writing papers. The most common bamboo species used for paper are

Dendrocalamus asper and *Bamboo bluemanea*. It is also possible to make dissolving pulp from bamboo. The average fibre length is similar to hardwoods, but the properties of bamboo pulp are closer to softwood pulps due to the broad fibre length distribution. With the help of molecular tools, it is now possible to distinguish the superior fibre-yielding species/varieties even at juvenile stagess of their growth, which can help in unadulterated merchandise production.

The rare fruits and young bamboo culms (i.e., the shoots) that sprout from many bamboo species are edible (Fig. 1.11). They are used in numerous Asian dishes and broths and are sold in various processed shapes in fresh, dried, and canned versions.

Fig. 1.11. Bamboo fruits and shoots (photos: W. Liese).

Bamboo charcoal is a new environment-friendly biomass material, which is a black porous solid product made from bamboo. In China and Japan, it is mainly used as fuel for cooking and drying tea. Most bamboo charcoal for fuel is briquette charcoal, and the rest is raw charcoal. Bamboo material has an extraordinary micro-structure: it has a high absorptive capacity after carbonization and becomes even more effective after activation. Moreover, bamboo charcoal can be used to purify water and eliminate organic impurities and odors.

Drinking water sterilized with chlorine can be treated with bamboo charcoal to remove residual chlorine and chlorides.

Bamboo vinegar is a sort of brown-black liquid made by condensing vapour and gas when bamboo has been carbonized. It contains more than 100 organic compounds such as saturated and unsaturated acid, alcohol, methanol, aldehyde, ketone, phenol, etc., and among them many components have a good capacity for sterilization and restraint. Its composition changes with collection and storage method. Because of its natural abundance of organic nutrients, organic bamboo vinegar is a method to pull out toxins from the body. Although acetic and formic acids are often found in bamboo vinegar, they are relatively mild compounds that display a variety of health benefits that especially aid in detoxification, sanitation and improving circulation.

1.2.4 Damage by fungi and insects

Bamboo has low natural durability (Liese and Kumar 2003). As a biological material, bamboo is in an endangering environment and susceptible to degradation by similar organisms which attack wood.

1.2.4.1 Damage by fungi

Damage of bamboo by fungi is divided into three main types: mildew, stain and rot. Therefore, the fungi contain three main types: mould fungi, stain fungi and rot fungi. In general, moulds belong to Deuteromycetes, stain fungi belong to Deuteromycetes or Ascomycetes, rot fungi belong to Basidiomycetes (Wang et al. 2000).

Freshly cut bamboo is susceptible to moulds and stain fungi due to the relative high content of carbohydrates in the tissue and the high moisture content. When bamboo is dried, moulds and stain fungi are inhibited. The occurrence of moulds and stain fungi differs according to climate, geographical area, and species. Twenty-two genera, 56 species have been identified from bamboo and bamboo products from 9 locations of China. The dominating fungal genera were *Trichoderma*, *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Botryodiplodia* (Ma and Jiang 2009). Fifteen species have been identified from moso bamboo in South China,

among which nine common fungi were *Alternaria alternata*, *Aspergillus flavus*, *Penicillium citrinum*, *P. chrysogenum*, *P. funiculosum*, *Chaetomium globosum*, *Fusarium pallidoroseum*, *F. camptoceras* and *Mucor hiemalis* (Wu and Wen 2000). 52 species have benn identified from bamboo in Yunnan Province of China, which belong to 4 subphyla, 6 classes, 10 orders, 18 families and 31 genera (Wang et al. 2000). Twenty five moulds have been isolated and identified and twenty of them are described from bamboo in the Hunan Province (Wu et al. 1994). Four moulds, *Alternaria viticola*, *Aspergillus restrictus*, *Cladosporium herbarum*, *Penicillium* sp., have been reported from *Phyllostachys pubescens* and *P. viridis* in the bamboo garden of Nanjing Forestry University, Jiangsu Province (Zhao et al. 1994).

Decay by rot fungi is a serious problem during storage and in use. The yield may be reduced by 25% during one year of storage by decay fungi. Various white-rot and some brown-rot fungi were found to attack bamboo stacks (Guha and Chandra 1979). *Trametes versicolor* and the ascomycete *Apiospora montagnei* were the two most degrading fungi causing mass loss of 21.6 % and 17.9 %, respectively (Kim et al. 2011). The development of fungal colonization and decay in the culms of *Gigantochloa scortechinii* during ground contact tests in a tropical soil were reported by Razak et al. (2002). After 24 months of exposure, untreated and ineffectively treated culms showed extensive decay. Fungi were observed by scanning electron microscopy (SEM) in the cell lumina, in the degraded cell walls and in the intercellular spaces. The morphology of decay was characteristic of white and soft-rot. In contrast, the tissues of culms with effective preservative treatments showed restricted hyphal colonization, infrequent hyphal invasion into cells via pits, or no cell wall degradation.

1.2.4.2 Damage by insects

Bamboo is susceptible to pest damage by beetles and termites, depending on the species and starch content. Because these occur even in dried bamboo and in interior usage, preservative treatment is necessary to ensure the durability. The bamboo felling season should be during the period of low carbohydrate content. In India, for example, this content is higher in spring than in winter, so it is recommended to cut between August and December to reduce the attack by insects and staining (Kumar et al. 1994). Immersing bamboo in water effectively prevents powder post beetle (*Dinoderus minutus* and *D. brevis*) infestation in cases of lower starch contents (Sulthoni 1990). The effectiveness of 11 commercial insecticides was tested by direct application to the beetles and by application to their food. Two environmentally

friendly insecticides, Cypermethrine and Permethrine, were the most toxic to the beetles (Varma et al. 1988).

The durability of species like *Bambusa pallida, B. balcooa, Dendrocalamus strictus* and *D. stocksii* against beetle attack was tested by the adult and larval inoculation technique. The results showed that preventing attack is highly difficult as the larvae dwell inside the culms with little exposure to the outside. In his context, fumigants like phosphines can be useful to penetrate the culms and kill the existing infestations (Remadevi et al. 2012).

Termites are the most aggressive organisms to the bamboo culms or finished bamboo products. As eusocial insects, termites live in colonies that number from several hundred to several million individuals. They are capable of utilizing cellulose as a source of food. Bamboo culms are rich in cellulose so they are easily attacked by termites causing severe damage. After a rapid deterioration, only a thin outer layer of the culm remains (Liese and Kumar 2003, Remadevi et al. 2005).

This vulnerability reduces the lifespan of bamboo products, causing a major hindrance to its applicability. Like with wood, bamboo can be safeguarded against deterioration (Liese 1959a, Liese and Kumar 2003) by protection and preservation practices during storage and use. Bamboo for overseas transport can be treated with a borax and boric acid solution by a short-term dipping or longer soaking period. An effective short-term protection against moulding, especially in the sensitive first 2 months after culm harvest, is by dipping the culms in solutions of 10% acetic acid (Tang et al. 2009) or 10% propionic acid (Tang et al. 2012).

1.3 Factors affecting fungal degradation tests

Several factors may influence bamboo degradation, for example site, growth rate, age, portion of bamboo, extractives content and the microenvironment (Hamaguchi 1953, Liese 1959a, 1985; Abdurachim 1964; Mohanan 1997; Kim et al. 2011; Suprapti 2010; Ma et al. 2010; Schmidt et al. 2011).

The behaviour of bamboo against decay fungi is an important parameter in bamboo establishment and use. Most investigations revealed that bamboo degradation is due to white and soft rot fungi, whereas brown-rot species were less aggressive. Among the investigated

fungi, *Coniophora puteana* and *Schizophyllum commune* varied with respect to the test method used: Low maximum mass loss was measured on agar under pure culture condition, whereas considerable degradation occurred in the `fungus cellar test´where the samples are placed in large metal containers on unsterile garden soil with different moisture accessibility (Schmidt et al. 2011). However, *C. puteana* and *S. commune* differed in decay activity: *C. puteana* produced maximum mass loss at low moisture content of 57 %, whereas decay by *S. commune* was highest at 182% moisture content (Schmidt et al. 2011).

Mixed microbial decay in soils with various water holding capacities was studied by Becker and Kaune (1966). Kaune (1970) and Worrall and Wang (1991) used for wood a vermiculite system with pure cultures of soft-rot fungi and proved its suitability for wood degradation tests. Adaskaveg et al. (1991) showed decay of palm wood by basidiomycetes with the vermiculite-block assay. Curling et al. (2002) exposed basidiomycetes to wood in a vermiculite-soil system.

Bamboo degradation was also influenced by treatment methods of bamboo. Heat treatment was performate by Leithoff (2011). Suhirman and Khusniati (1987) studied the durability of bamboo by mudsubmission treatment.

When comparing data, another difficulty is due to the different tests performed, such as field and laboratory tests. Also within the same test, the different standards (Asia, European and American) may differ by sample size, duration of tests and fungal strains. Decay tests for white-rot fungi on bamboo were performed in 12 weeks according to the American Wood Preserves Association Standard E10-01, while the soft-rot tests was conducted for 20 weeks using a modified vermiculite burial test (Kim et al. 2011).

1.4 Modern techniques to study the morphology of degraded bamboo

The knowledge on bamboo degradation by white-rot, brown-rot and soft-rot fungi has been enlarged considerably in recent years (Cho et al. 2008, Kim et al. 2008, Xu et al. 2013). From the current research technologies and published literature, six modern methods were used to study fungal bamboo degradation. They are summarised in the following sections.

1.4.1 Scanning electron microscopy (SEM)

A scanning electron microscope (SEM) produces images by scanning the sample with a focused beam of electrons. The various signals which include information with the sample's surface topography and anatomy can be produced and detected when the electrons act with atoms. In general, the electron beam is scanned in a raster pattern, and the image can be produced when the beam's position is mixed with the detected signal. SEM can gain resolution better than 1 nanometre. Specimens can be discovered in various conditions, such as high vacuum, low vacuum, or (with the environmental SEM technique) wet factors.

Using SEM, it was found that attacks to parenchyma cells and places near the inner skin of bamboo were the most frequent and the vessels were the primary paths for the spread of mycelium in the bamboo (Xu et al. 2013).

1.4.2 Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy can afford high-resolution optical images with depth selectivity (Pawley 2006). The ability to obtain in-focus images from selected depths is the most important character of CLSM, which is known as optical sectioning. The images are obtained point-by-point and reconstructed with computer so that three-dimensional reconstructions of topologically complex objects can be observed. Marvin Minsky patednted the principle of confocal microscopy in 1957, however, the developments of lasers for CLSM become to standard technique untill the 1980s (Pawley 2006).

The parenchyma cells of *Phyllostachys pubescens* after degradation by white rot fungus *Lentinula edodes* was observed by CLSM. The results showed that the preferential degradation of the culm by *L. edodes* was mostly confined to the parenchyma cells at the early stagess (Kim et al. 2008). Cho et al 2008 investigated *P. pubescens* degraded by the brown-rot fungus *Gloeophyllum trabeum* and demonstrated that the narrow layers are resistant against brown-rot attack and the main degradation occurred in the broad layers.

1.4.3 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is a method in which a beam of electrons is transmitted through an ultra-thin specimen and interacted with the specimen as it passes through. The image is produced by the interaction of the electrons transmitted through the sample. Furthermore, the image is magnified and focused on an imaging device, such as a fluorescent screen on a layer of photographic film, or by a sensor such as a CCD camera can be discovered.

Due to the small de Broglie wavelength of electrons, the resolution of TEM is obviously higher than light microscopes. Therefore, the user can determine fine details even with a single column of atoms, which is thousands of times smaller than the smallest resolvable object in a light microscope.

Phyllostachys pubescens degraded by the brown-rot fungus *G. trabeum* (Cho et al. 2008) and by *L. edodes* (Kim et al. 2008) were examined by TEM, both results showed that the compound middle lamellae in bamboo fibres were degraded at early stages of decay.

1.4.4 Ultraviolet microspectrophotometry (UMSP)

UMSP is a method to measure the relative lignin content semi quantitatively and to get information on the lignin structural units. The technique is based on the ultraviolet illumination of thin transverse sections. Lignin displays a characteristic ultraviolet absorbance spectrum with absorbance maxima around 280 nm due to the presence of associated phenylpropane groups with several chromophoric structural elements (Fergus et al. 1969, Scott et al. 1969, Fergus and Goring 1970a, b, Sarkanen and Hergert 1971, Bauch et al. 1976, Hesse et al. 1991). No other component of the mature cell wall displays ultraviolet absorbance properties in the same spectral region, and therefore the intensity of absorbance may be related to the concentration of lignin across the cell wall.

Koch and Kleist (2001) studied the S_2 layer of fibre wall of *Phyllostachys edulis*. They showed the lamellar structure with increasing lignin content from the lumen towards the middle lamella and observed a typical UV spectrum for bamboo fibres with a guaiacyl peak at 280 nm and a shoulder between 310 - 315 nm, which can be linked to the presence of *p*-

coumaric acid esters. Furthermore, discrimination between the types of lignin is possible due to different ratios of their guaiacyl- and syringylpropane units.

Kim et al. (2008) investigated the changes in lignin content of *P. pubescens* after degradation by *L. edodes*. The lignin concentration in the bamboo fibres gradually increased from the inner part (lumen side) to the outer part. The lowest absorbance in fibre walls was always associated with the wall regions adjacent to the lumen, whereas high absorbance was recorded in the regions close to the middle lamellae.

Phyllostachys pubescens degraded by the brown rot fungus *G. trabeum* showed that the absorbance maxima of the compound middle lamella region (CML), including the cell corner developed at 312 - 315 nm. The result underlines that the CML in bamboo is mainly composed of *p*-hydroxyphenyl lignin (Cho et al 2008).

1.4.5 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy combines microscopy and imaging techniques for identification and localization. Generally, FTIR spectroscopy is employed to determine the chemical composition of organic compounds. By combining FTIR spectroscopy with microscopy, the chemical FTIR analysis gains local resolution. The development of focal plane array (FPA) detectors and acceleration of measurement times from days to minutes have made it possible to image the distribution of chemical compounds in biological materials (Salzer et al. 2000). FPA detectors consist of an array of several thousand detector elements. These detector elements simultaneously record spectra, which can be converted into images displaying the spatial distribution of the chemical properties of a sample.

FTIR spectroscopy has successfully been used for identification of bacteria and also a few fungi, both at genus and species level (Mariey et al. 2001, Naumann et al. 1991, Ngo-Thi et al. 2003, Naumann et al. 2005). In addition, the lignin distribution in cross sections of beech and poplar was analysed and the spatial resolution compared using FTIR spectroscopy (Naumann and Polle 2006).

The changes in lignin content of *P. pubescens* after degradation by *L. edodes* were estimated by FTIR spectra. The absorption bands assigned to GS (1511 cm^{-1}), S (1127 cm^{-1}), SH (834

cm^{-1}) and HGS (1166 cm^{-1}) lignin units (Faix 1991) were significantly decreased. The absorption band at 1650 cm^{-1}, assigned to carbonyl groups (Faix et al. 1991), was also significantly decreased. His finding can be interpreted that lignin was more degraded by *L. edodes* than carbohydrates (Kim et al. 2008). In particular, the band at 1646 cm^{-1}, which was assigned to conjugated carbonyl groups originating from lignin, significantly decreased, which suggested that not only H-unit lignin but guaiacyl-type or syringyl-type lignin in *P. pubescens* were attacked by *G. trabeum* (Cho et al 2008). Significant changes in FTIR spectra could be seen after decay by *Phanerochaete chrysosporium* (white-rot) and *G. trabeum* (Xu et al. 2013). Therefore, FTIR can be applied to investigate changes in the chemical structure of bamboo before and after decay by white-rot and brown-rot.

1.4.6 X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy has been successfully used for chemical analysis of various materials. Using XPS, a surface layer is analyzed, and the elemental composition of the samples is examined. XPS can also disclose information about the chemical bonding environment of the elements. Furthermore, the percentage of certain chemical components on a surface can be determined.

In pulp and paper research, XPS demonstrates the energy distribution of photoelectrons ejected from the inner shells of atoms by a soft X-ray, which is a measurable investigation technology to study the surface chemistry of complex organic materials (Johansson and Cambell 2004, Li and Reeve 2004, Fardim et al. 2005). XPS has been used for the study of the surface of red oak and red pine extracted wood (Nzokou and Kamdem 2005), the surface of different wood species (Sinn et al. 2001) and wood chemical composition after heat-treatment (Inari 2006). The changes of rice straw lignin and cellulose after decay by white-rot and brown-rot fungi were studied by XPS (Dey et al. 1992). The result demonstrated that the XPS data could be related with the loss of lignin and polysaccharides of straw, which is degraded by fungi and determined by chemical analyses.

Xu et al. (2013) investigated *Phyllostachys edulis* decayed by *P. chrysosporium* and *G. trabeum* using XPS spectroscopy and verified that this method can be applied to study the changes in the chemical structure of bamboo before and after decay by rot fungi.

1.5 Aim of the study

Bamboo is a fast growing woody grass of increasing interest for the sustainable production of materials with many applications for buildings and industrial utilization. In general, bamboo has generally a low natural durability and is easily attacked by fungi during storage, transport and final use. However, little is known about the fungi inhabiting and degrading the bamboo. Furthermore, for applications it is important to know which fungi might cause harm to potential products.

Like other lignocellulose materials, bamboos are subject to biodegradation by fungi under particular conditions which affect their quality (Hamid et al. 2003). The resistance of bamboo against decay fungi serves as an important parameter in bamboo utilization. Several factors may influence the natural durability of bamboo, such as growth site, growth rate, age, portion of bamboo, extractives content and the microenvironment.

When comparing data, another difficulty is due to the different tests performed, such as field and laboratory tests. Also within the same test, the different standards (Asia, European and American) may differ by sample size, duration of tests and fungal strains. Therefore, it is important to test the bamboo species using the same standards.

The overall objective of this study is to identify bamboo-inhabiting fungi and evaluate their damage to the substrates. The study consists of the following specific objectives:

(1) to isolate and identify some bamboo-inhabiting fungi by molecular methods,
(2) to test bamboo degradation by different methods,
(3) to analyze chemical changes of bamboo tissue after degradation,
(4) to study micromorphological cell wall characteristics after degradation.

The results obtained in this work, together with other data reported in literature on the decay of bamboo (Liese 1959a, 1985, Banerjee and Mukhopathya 1962, Purushotham 1963. Abdurachim 1964, Wang and Hsieh 1968, Murphy et al. 1991, Leithoff and Peek 2001, Razak et al. 2002, 2006, Hamid et al. 2003, Zhang et al. 2007, Kim et al. 2008, 2011, Suprapti 2010, Ma et al. 2010, Schmidt et al. 2011, Wei et al. 2012) may contribute to the characterization and appreciation for bamboo and bamboo products. Such characterization is important not only for its correct utilization but also for improved market promotion.

2 Material and Methods

2.1 Molecular identification of bamboo inhabiting fungi

Bamboo is easily attacked by fungi during storage, transport, processing and final use. Little is known about the fungi inhabiting and degrading it. Therefore, those fungi were isolated. Fungi were identified by molecular techniques because the classical methods may fail to identify morphologically similar organisms.

2.1.1 Collection of infected bamboo samples

The fungus-infected samples were obtained with the help of Prof. Dr. Walter Liese from his worldwide colleagues and derived from eight countries, Ethiopia, China, Costa Rica, Germany, Indonesia, Philippines, Thailand and Vietnam. The samples were mostly gained from culms for practical usage, so that the bamboo species is only known in a few cases, such as *Bamboo multiplex* from Thailand, *Dendrocalamus asper* from Indonesia and Thailand, *D. brandisii* from Costa Rica and *Phyllostachys glaucoviridescens* from Germany. Fig. 2.1 shows moulded bamboo after sea transport to Germany.

Fig. 2.1. Moulded bamboo after ship transport from Asia (right photo: T.K.H. Tang).

2.1.2 Isolation and purification of fungi

The fungi were isolated from the culm surface by streaking spores or hyphae with an inoculation needle and were cultivated on the 2 % malt extract agar (MEA, Oxoid) at room temperature for approx. 5 days and then subcultured on agar plates till pure cultures.

2.1.3 DNA extraction and amplification by polymerase chain reaction (PCR)

DNA was extracted from the mycelia with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) was performed with the Qiagen Tag Core Kit in the PTC-100 thermocycler (MJ Research, Watertown, MA, USA). To amplify the rDNA-ITS region, the standard primers ITS1 and ITS 4 were used (White et al. 1990).

Sequences of used primers are:
ITS1: 5'-TCCGTAGGTGAACCTGCGG-3' (forward)
ITS4: 5'-TCCTCCGCTTATTGATATGC-3' (reverse)

The PCR protocol was as follows: initial denaturing at 98 ℃ for 4 min, followed by 35 cycles of 94 ℃ denaturing, 55 ℃ annealing and 72 ℃ extension, each for 1 min. The final extension was at 72 ℃ for 7 min. The PCR products were stored at 8 ℃ (Schmidt et al. 2002).

2.1.4 PCR products examination and purification

The PCR products were examined prior to sequencing using electrophoresis on 2 % agarose gels (DNA agarose, Biozym, Hess. Oldendorf, Germany) in 0.5 TBE buffer for 30 min with the Mupid-ex U system (Advance, Tokyo, Japan). Gels were stained with GelStar Nucleic Acid Gel Stain (Cambrex, Rockland, ME, USA) and examined using UV transillumination.

PCR products assumed to be suitable for sequencing were purified using the QIAquick PCR Purification Kit (Qiagen).

2.1.5 Sequencing and sequence analysis

Sequencing of the purified PCR products was performed for both DNA strands by Eurofins MWG Operon (Ebersberg, Germany). The complete rDNA-ITS sequence was obtained by overlapping both strands. Once a sequence has been obtained for a sample in question, it can be identified by comparison with sequences deposited in the international databases. The NCBI Genbank (http://www.cnbi.nlm.nih.gov/) is one of a public database which is used worldwide. To find the most identical sequences, the Basic Local Alignment Search Tool (BLAST) was used identification by comparison.

The whole procedure is demonstrated in Fig. 2.2.

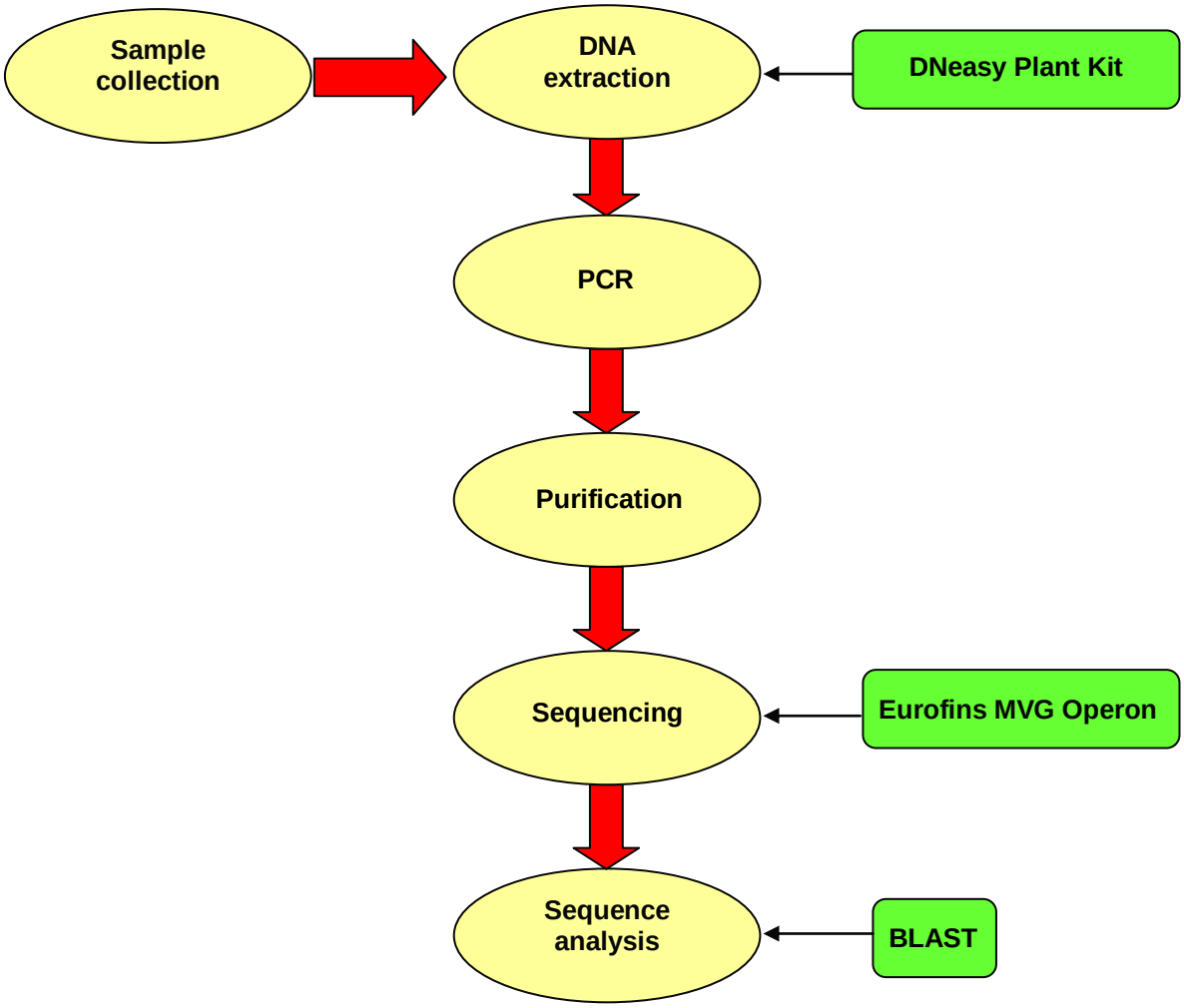

Fig. 2.2. Procedure for molecular identification of bamboo inhabiting fungi.

2.2 Identification of *Penicillium* and *Aspergillus* isolates from bamboo

Aspergillus and *Penicillium* are the two most important mould genera which inhabit harvested bamboo culms. However, both genera contain very closely related species of which some also have various synonyms. This may lead to uncertain results when only the rDNA-ITS sequences are used for identification. Furthermore, the international DNA databases are man-made and contain mistakes, such as misidentifications. The following experiments were done in the Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, Institute of the Royal Netherlands Academy of Arts and Sciences.

2.2.1 Morphological identification of *Penicillium* and *Aspergillus* isolates

Classically, *Penicillium* and *Aspergillus* are identified by morphological characters. *Penicillium* and *Aspergillus* isolates obtained from bamboos were inoculated in a three point position on Czapek yeast extract agar (CYA), yeast extract sucrose agar (YES), creatine agar (CREA) and malt extract agar (MEA, Oxoid). All media were prepared according to Samson et al. (2010). Colony characteristics were recorded after 7 d of incubation at 25 or 37 ℃.

2.2.2 Molecular identification of *Penicillium* and *Aspergillus* isolates

The rDNA-ITS-sequence within a species is highly conserved but differs even between closely related species. Sequencing these can allow an unambiguous identification, respectively classification of a fungus. *Aspergillus* and *Penicillium* contain very closely related species of which some also have various synonyms. This may lead to uncertain results when only the ITS sequence is used for identification. Therefore, *ß*-tubulin and calmodulin sequences were obtained for these fungi and also used for BLAST identification. The DNA regions were amplified with the following PCR primers.

ITS (see 2.1.3),

β-tubulin:
T10: 5’-ACGATAGGTTCACCTCCAGAC-3’ (forward),
BT2b: 5’-ACCCTCAGTGTAGTGACCCTTGGC-3’ (reverse),

calmodulin:
CMD5: 5’-CCGAGTACAAGGARGCCTTC-3’ (forward),
CMD6: 5’-CCGATRGAGGTCATRACGTGG-3’ (reverse).

2.3 Blue-stain test

2.3.1 Used blue-stain fungi

The 11 strains of blue-stain fungi used are listed in Table 2.1, of which 9 strains are our own isolates from bamboo and 2 derive from the institute collection.

Table 2.1. Used blue-stain fungi

Species	Laboratory coding	Origin
Alternaria alternata	D84-2	Bamboo
Alternaria tenuissima	D82-3-2	"
Aureobasidium pullulans	87	Institute
Botryosphaeria subglobosa	D26	Bamboo
Cladosporium cladosporioides	D16-2	"
C. cladosporioides	D81-2	"
Epicoccum nigrum	D20	"
Hormonema dematioides	85	Institute
Pestalotiopsis microspora	D24	Bamboo
Phoma macrostoma	D64-1	"
Unidentified isolate	1.5	"

2.3.2 Investigated bamboo

Investigations were conducted on culm sections of the bamboo species *Bambusa maculata, Gigantochloa atroviolacea and Phyllostachys pubescens. Bambusa maculata* and *G. atroviolacea* originating from Indonesia were obtained from CONBAM (Geilenkirchen, Germany) and *P. pubescens* originating from Germany were obtained from the Bamboo Centre (Baden Baden, Germany). The curcuma-test (Peylo 2001) was applied to ensure that the samples were not contaminated with boron compounds which are commonly used for preventing mould during storage and transport. The bamboo specimens were cut into samples of $3 \times 1 \times$thickness cm^3.

2.3.3 Blue-stain test set-up

The experiment was performed with pure cultures of blue-stain fungi (Table 2.1) in Petri dishes (9 cm diameter). The culture medium consisted of 2 % malt extract (Merck) and 1.5 % agar (Bacto) in distilled water. The Petri dishes were inoculated with fresh mycelium-agar plugs (8 mm^2) from pre-cultures and incubated at room temperature for 14 days. When the agar surfaces were completely covered by mycelium, three bamboo samples sterilized in the autoclave at 121 °C for 30 min were placed on the mycelium in each dish. Three dishes were used for each strain. After 4 and 20 weeks of incubation at 20±2 °C and 65±5 % relative humidity (RH) in a climate room, harvests were made and the mycelium was removed from the bamboo sample surface. Samples were then cut into small specimens and put into PEG 2000 (polyethylene glycol 2000) (20 ml PEG plus 60 ml distilled water) for infiltration. After 1 week water evaporation period, specimens were cut into 20 µm sections with a microtome. Since bamboo samples easily break during microtoming, sections were placed on adhesive plaster. The cut sections were embedded in glycerol, examined with a light microscope Olympus BX51 and photographed.

2.3.4 Assessment of hyphal growth and chlamydospores in bamboo

The development of hyphal growth and chlamydospores on/in the sample was assessed according to the scheme in Table 2.2, which values the percentage of the tissue covered with mycelium and the density of clamydospores in vessels and parenchyma cells.

Table 2.2. Evaluation scheme hyphal growth and chlamydospores on bamboo

Coverage of tissue	Description	Density of chlamydospores	Description
0	No	0	No
1	1 - 10 %	1	1 - 10 %
2	11 - 25 %	2	11 - 25 %
3	26 – 50 %	3	26 – 50 %
4	> 50 %	4	> 50 %

2.4 Degradation tests

2.4.1 Preserving jar test

2.4.1.1 Used fungi

Eight strains are used, which belong to brown-rot (2 strains), white-rot (4 strains) and soft-rot (2 strains) fungi. Details are listed in Table 2.3.

Table 2.3. Used fungi

Rot type	Species	Laboratory coding	Other codings/ use for EN
Brown	*Coniophora puteana*	167	
	Gloeophyllum trabeum	183	Ebw. 109, EN 113
White	*Pleurotus ostreatus*	11	ATCC 44737
	Trametes versicolor	63	CTB 863A, EN 113
	Schizophyllum commune	87	
	S. commune	98	
Soft	*Chaetomium globosum*	10	ATCC 44753
	Paecilomyces variotii	13	ATCC 44741

2.4.1.2 Investigated bamboo and wood samples

Investigations were conducted on culm sections of *Bambusa maculata* and *Gigantochloa atroviolacea* from Indonesia and *Phyllostachys pubescens* from Germany. The curcuma-test (Peylo 2001) was applied to ensure that the samples were not contaminated with boron. Samples (3×1× thickness cm^3) were dried at 103 ℃, weighed, and autoclaved. Wood samples (5×2.5×1.5 cm^3) from *Fagus sylvatica* L. and *Pinus sylvestris* L. were used as controls according to EN 113.

2.4.1.3 Preserving jar test set-up

Household preserving jars with a volume of 500 ml were autoclaved for 20 min and used as culture vessels (Schmidt 1986). Culture medium for the Basidiomycetes consisted of 110 ml of 2 % malt extract and 1.5 %. For the Ascomycetes, Abrams agar (Savory 1954) was used, supplemented with 0.1 % yeast extract as a vitamin source. The jars were inoculated with fresh mycelial agar plugs (8 mm^2) and incubated at 21 ℃ and 70 % RH for 1, 3, and 12 months, respectively. For each harvest time, two parallels were used to consider possible contaminations during the long cultivation time (Fig. 2.3).

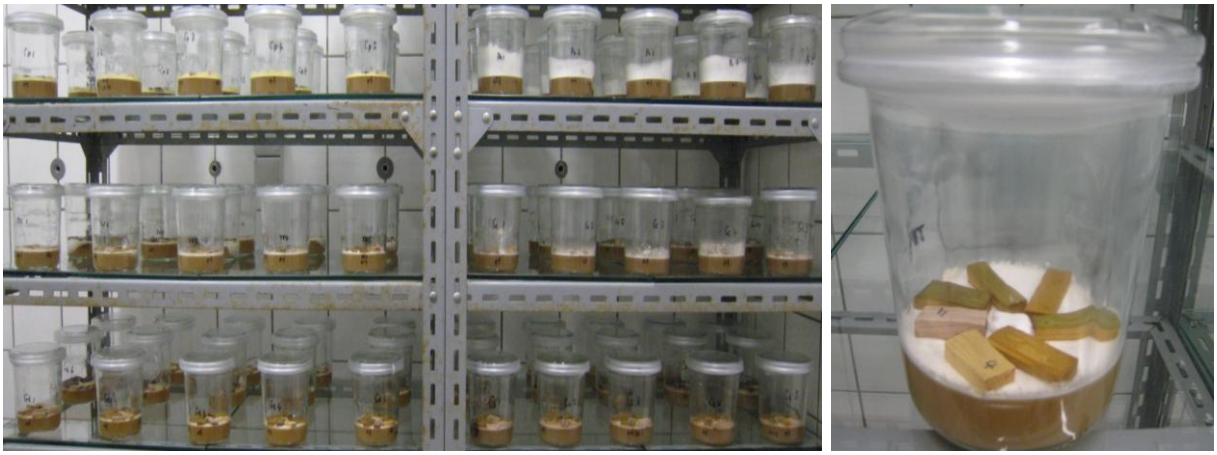

Fig. 2.3. Preserving jar test set-up.
Left: Preserving jars in the incubation room; right: samples with mycelium of *Trametes versicolor* after 1 week incubation.

2.4.1.4 Assessment of degradation in preserving jars

Evaluation of mass loss of bamboo and control wood samples decayed by fungi was done after 1, 3 and 12 months of incubation. At harvest, the mycelium was removed from the sample surface; the samples were weighed for final wet weight and oven dried at 103 ℃ for 3 days. The mass loss (ML) due to fungal decay was determined according to the equation:

$$\text{Mass loss}(\%) = \left(\frac{M_1 - M_2}{M_1}\right) \times 100$$

M_1: dry weight before fungal degradation
M_2: dry weight after fungal degradation.

The standard deviation for each 4 specimens per treatment group was calculated according to ANOVA, SPSS software 2002.

2.4.2 Fungus cellar test

2.4.2.1 Used fungi

Two species were used: the brown-rot fungus *Coniophora puteana* 167 from the laboratory collection and the white-rot fungus *Schizophyllum commune* 87 from bamboo (own isolation).

2.4.2.2 Investigated bamboo

The seven bamboo species tested are listed in Table 2.4. Culm sections were obtained from CONBAM and the Bamboo Centre, Baden-Baden. Sections were proved with the curcuma-test for boron (Peylo 2001). Sections (25 cm) were longitudinally halved, dried at 103 °C, weighed, dipped in tap water for two days and autoclaved at 121 °C for 40 min.

Table 2.4. Investigated bamboo

Bamboo species	Local name	Culm diameter (cm)	Origin
Arundinaria amabilis	Tonkin	3.5-4.0	Vietnam
Bambusa maculata	Tutul	4.5-5.5	Indonesia
Dendrocalamus asper	Petung	13.5-14.0	Indonesia
Gigantochloa atroviolacea	Wulnung	5.5-7.0	Indonesia
Phyllostachys nigra	Nigra	2.0-2.5	Japan
P. nigra 'Boryana'	Boryana	3.0-4.0	China
Phyllostachys pubescens		5.5-6.5	Germany

2.4.2.3 Fungus cellar test set-up

Metal tubs (120 cm long, 60 cm wide) were filled with 30 L unsterile compost soil from the institute garden (Gersonde and Becker 1958). Bamboo samples were placed either on autoclaved wood supports or directly on the soil (Fig. 2.4). Each sample was infected with three small inoculation wood pieces (0.5 cm^3) containing mycelium. The tubs were covered with panes of glass and kept at 23 °C and 90 % RH for 1 year. The soil was moistened weekly with sprayed tap water. The development of fungi on the samples was observed by visual inspection. After harvest, mass loss and final moisture content of samples were measured and evaluated.


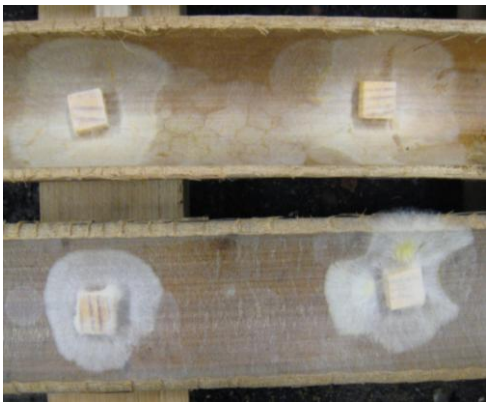

Fig. 2.4. Fungus cellar test set-up.
Left: samples in the Fungus cellar; right: samples with mycelium of *Schizophyllum commune* after 1 week of incubation.

2.4.3 Kolle flask test

2.4.3.1 Used fungi

The 10 strains of white-, brown- and soft-rot fungi used derived from the laboratory strain collection and are listed in Table 2.5.

Table 2.5. Used fungi

Rot type	Species	Laboratory Coding	Other codings/ use for EN
Brown	*Coniophora puteana*	1	Ebw. 15/ EN 113
	C. puteana	159	
	C. puteana	167	
	C. puteana	247	
	Gloeophyllum trabeum	183	Ebw. 109/ EN 113
	G. trabeum	259	
White	*Trametes versicolor*	63	CTB 863/ EN 113
	Schizophyllum commune	87	
Soft	*Chaetomium globosum*	10	ATCC 44753
	Paecilomyces variotii	13	ATCC 44741

2.4.3.2 Investigated bamboo and wood samples

The five bamboo species tested are presented in Table 2.6. Culm sections from CONBAM and the Bamboo Centre were previously proven for boron. Samples were prepared by cutting to dimensions of 5 cm (length) × 2.5 cm (width) × 0.5-3.5 cm (wall thickness). Wood samples from *F. sylvatica* and *P. sylvestris* were used as controls according to EN 113. All samples were put into a climate room at 20±2 °C and 65±5 % RH for 4 weeks, weighed, wrapped in plastic (Sengewald Flexopeel, Germany) and sent to the BBF sterilization service (Kernen, Germany) for gamma radiation.

Table 2.6. Investigated bamboo

Bamboo species	Local name	Origin
Bambusa maculata	Tutul	Indonesia
Dendrocalamus asper	Petung	Indonesia
Gigantochloa atroviolacea	Wulung	Indonesia
Guadua angustifolia	Guadua	Columbia
Phyllostachys pubescens	Moso	China
P. pubescens		Germany

2.4.3.3 Durability test

The durability experiment was performed with fungal pure cultures in Kolle flasks with white-, brown-, and soft-rot fungi according to the European standards EN 350-1 (1996), EN 350-2 (1997) and EN 113 (1996). The culture medium consisted of 2 % malt extract (Merck) and 1.5 % agar (Bacto) in distilled water. Flasks containing 48 ml medium were plugged with cotton, sterilized in the autoclave at 121 °C for 30 min, inoculated with a mycelial plug of the test fungus and incubated until mycelial growth covered the surface of the medium (Fig. 2.5). Each two gamma ray sterilized samples were put aseptically in one flask and each three flasks were used for one fungal strain, with altogether 480 Kolle flasks. After 16 weeks of incubation at 20±2 °C and 65±5 % RH for the brown-rot and white-rot fungi and 28±2 °C and 65±5 % RH for the soft-rot fungi, mycelia were removed from the sample surface, the samples were weighed for final wet weight determination and oven dried at 103 °C for mass loss (ML) determination. Percentage of ML was calculated by weight comparison before and

after incubation. Statistical differences were analyzed using variance analysis (ANOVA, SPSS software 2002).

Fig. 2.5 shows some cultures with *P. pubescens* in Kolle flasks.

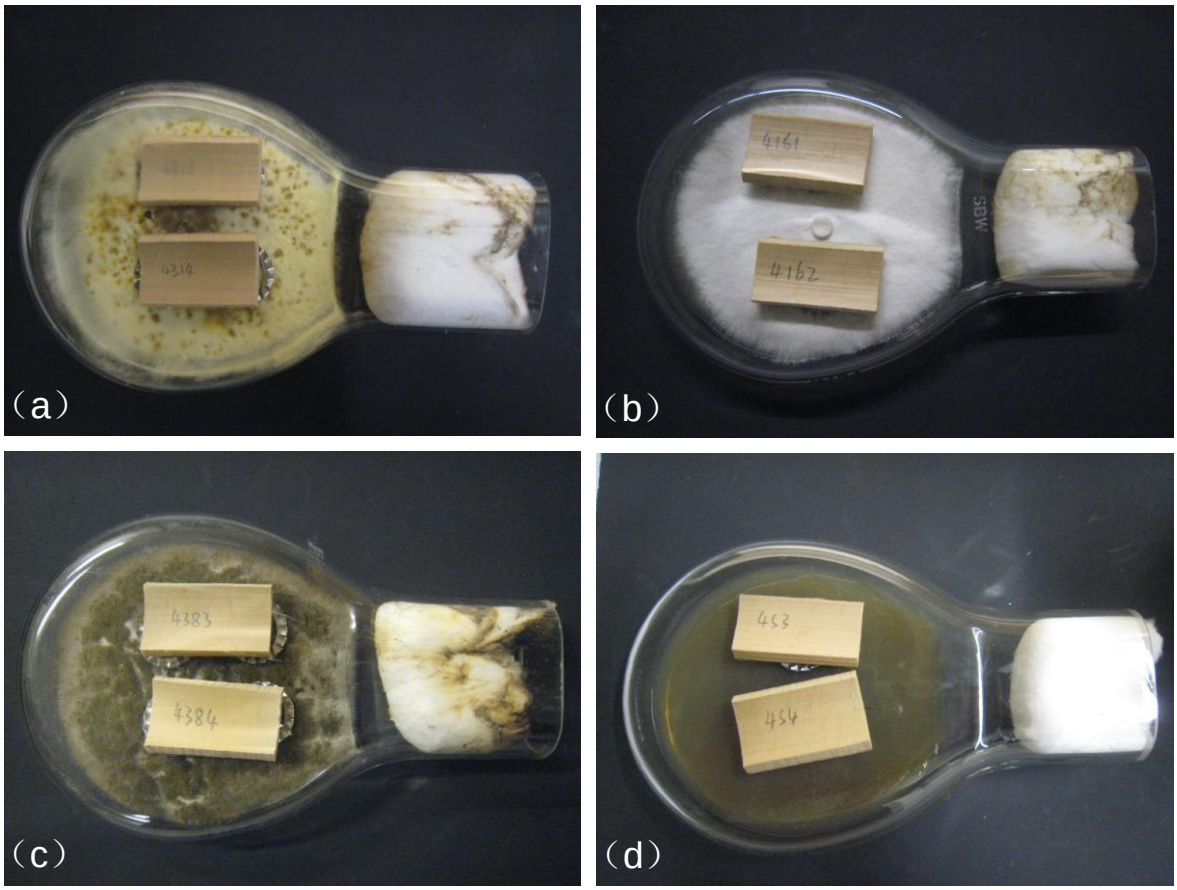

Fig. 2.5. Kolle flask test set-up.
(a): *Coniophora puteana*; (b): *Trametes versicolor*; (c): *Chaetomium globosum*; (d): control.

2.4.3.4 Assessment of fungal growth and classification of bamboo durability

The development of fungal growth on the specimens was valued by the density and the percentage of the sample surface covered with mycelium. Based on the average mass loss, bamboo durability against fungi was correlated to durability classes considering EN 350-1 (1996), Abdurachim (1975) and Djarwanto and Suprapti (2004).

2.4.4 Vermiculite test

2.4.4.1 Used fungi

The brown-rot fungus *Coniophora puteana* strain 167 from the laboratory collection and the white-rot fungus *Schizophyllum commune* strain 87 from bamboo were held on agar plates of 2 % malt extract (Oxoid) and 1.5 % agar (Oxoid).

2.4.4.2 Investigated bamboo and wood samples

Phyllostachys pubescens (diameter 5.5-6.5 cm) and *Gigantochloa atroviolacea* (diameter 5.5-7.0 cm) from CONBAM and the Bamboo Centre proven for boron were used. Culm sections were cut to 5 cm length, 2.5 cm width and 0.5–3.5 cm wall thickness. Wood samples of *F. sylvatica* and *P. sylvestris* were cut to 5 cm length, 2.5 cm width and 1.5 cm height. Samples were put into a climate room at 20±2 °C and 65±5 % RH for 4 weeks, weighed, wrapped in plastic (Sengewald Flexopeel) and send for gamma radiation to the BBF sterilization service. To calculate the initial weight, moisture content at 20 °C and 65 % was adjusted downward.

2.4.4.3 Vermiculite test set-up

The decay experiment was performed in 500 ml preserving jars (Fig. 2.6). Vermiculite of 2-3 mm particle size was obtained from the Vermiculite-shop (thinex new media, Dortmund, Germany, www.vermiculit-shop.de) and sieved through a 2 mm sieve. Each preserving jar was filled with 200 ml vermiculite. Tap water or 2% malt extract solution were added in 80, 100, 120, 140 and 160 ml amounts per jar. The jars covered with glass lids were sterilized in the autoclave at 121 °C and 2.1 bar for 30 min. Each two bamboo or wood samples were put aseptically in one jar. Two jars were used for one fungus. Vermiculite and sample were separated by a metal ring to avoid liquid diffusion in the sample. A plug of the test fungus from agar plates was then placed on the sample top. After 32 weeks of incubation at 20±2 °C and 65±5% RH, the mycelium was removed from the sample surface, the samples were weighed for final wet weight and oven dried at 103 °C for 3 days for ML determination. ML loss was calculated by weight comparison before and after incubation. Standard deviation for each 4 specimens per treatment group was calculated according to ANOVA, SPSS software 2002.

Fig. 2.6. Vermiculite test set-up.
Left: vermiculite jars in the incubation room; right: preserving jar with vermiculite and bamboo samples.

2.4.4.4 Assessment of fungal growth and vitality test after incubation

The development of fungal growth on the samples was assessed according to the scheme in Table 2.7, which values hyphal density and percentage of sample surface covered with mycelium. After 32 weeks of incubation, small portions of overgrown vermiculite were transferred from the jars to malt agar plates to prove the vitality of the mycelium after the vermiculite test.

Table 2.7. Evaluation scheme for hyphal growth on bamboo and wood samples

Hyphal density	Description	Sample coverage	Description
0	no growth	0	no coverage
1	sparse mycelium	1	1 – 33 % coverage
2	normal mycelium	2	34 – 66 % coverage
3	thick mycelium	3	67 – 99 % coverage
		4	total coverage

2.4.5 Field test

2.4.5.1 Investigated bamboo

A small field test was done with culm sections of 4 bamboo collectives (Table 2.8) obtained from Rudolf Schachtrupp, Hamburg, Germany. Sections were proven for boron.

Table 2.8. Investigated bamboo

Bamboo	Geographical origin	Culm diameter (cm)	Length after sawing (cm)
Bambusa vulgaris	India	6.0 - 6.5	20
unknown species 3	China	5.5 – 6.0	30
unknown species 5	China	2.0 - 3.5	20
unknown species 10	Thailand	3.0 - 3.5	28

2.4.5.2 Field test set-up

The bamboo-stake specimens were exposed to soil near the greenhouse of the Thünen-Institute (vTI) in Hamburg-Lohbrügge, in March 2009. The specimens were put by 1/2 of their length into the soil (Fig. 2.7).

Fig. 2.7. Field test set-up.

2.4.5.3 Assessment of stake test

After 3.5 years incubation in field the bamboo samples were harvested, the soil and moulds removed from the sample and cut into two parts: in ground and above ground. Each part was cut into three sections: top, middle and bottom. To assess the natural durability in the field, the decay of the different sections were classified visually into 5 classes according to the rating scheme of Papadopoulos (2010). The classification is shown in Table 2.9.

Table 2.9. Evaluation scheme for stakes in the field

Rating	Description
0	total decay
1	severe decay
2	moderate decay
3	slight decay
4	no decay

2.5 Chemical analysis of bamboo after degradation

2.5.1 Degraded bamboo samples

Degraded bamboo samples from the preserving jar test (Chapter 2.4.1) of the 12 months incubation were used. The samples were splintered and ground in a mill (MF 20 Basic, IKA Werke GmbH, Germany), producing particles passing a 10-mesh screen.

2.5.2 Acetone and ethanol extraction

The aim of extraction is to remove extractives, including possibly sugar monomers and dimers, and phenolic glycosides. After measuring the moisture content of samples, the extractions were conducted with an accelerated solvent extractor (ASE 200, Dionex) (Fig. 2.8). About 1 g of milled degraded bamboo was extracted by acetone:water (9:1) and ethanol:water (8:2), respectively. The used extraction parameters are listed in the Table 2.10.

Table 2.10. Extraction parameters

	Acetone/water	Ethanol/water
Preheat phase [min]	5	5
Heating phase [min]	5	5
Static phase [min]	10	10
Purgeing with N [sec]	90	90
Cycles	2	2
Pressure [bar]	100	100
Temperature [℃]	70	70

The total extractive content was determined by adding together the single extraction values.

Fig. 2.8. ASE 200 Accelerated Solvent Extractor.

2.5.3 Hydrolysis

Degraded bamboo samples were ground in a vibration mill (Herzog Maschinenfabrik, Germany). All samples were climatized. The moisture content was measured and the results were taken into account. Two step hydrolysis methods were used.

For pre-hydrolysis, 2 ml 72 % H_2SO_4 were added to 200 mg (+/- 10 mg) (atro) the sample. The samples were hydrolyzed for 1 h at 30 ℃ (incubated in a water bath). The reaction vessel was a short test tube. The sample was stirred during the reaction. After 1 h the reaction was stopped by addition of 6 ml distilled water. Each sample was transferred into a 100 ml

volumetric flask using 50 ml of distilled water. The vessel was closed with a little condenser (a glass ball).

For post-hydrolysis, samples were hydrolyzed under pressure (0.12 mPa) in an autoclave for 40 min. at 120 ℃. After cooling down to room temperature, the flasks were filled up with distilled water to exactly 100 ml and were shaken. Thereafter, the condensed lignin residue was removed by filtration over a G4 sinter glass crucible. One ml of the liquid was transferred into a sample vial for the analysis with the borate system and 1.5 ml was saved in a plastic tube for second analyses. This sample was frozen. The filtered lignin was washed intensively with distilled water. After this the crucible was dried at 105 ℃ and the lignin content was determined gravimetrically.

2.5.4 HPAEC-borate analysis

The high-performance-anion-exchange chromatography-borate analysis was performed by using a 5.0 mm bore column of 115 mm length (Omnifit) filled with the strong anion exchange resin (MCI Gel CA08F (Mitsubishi) at 65 ℃) (Fig. 2.9). The mobile phase (0.7 ml min^{-1}) was made of A: 0.3 M potassium borate buffer pH 9.2 and B: 0.9 M potassium borate buffer pH 9.2. After sample injection, the separation was started with 90 % A and 10 % B. A linear gradient was run within 35 min to 10 % A and 90 % B. Data acquisition was stopped after 43 min. Sugar quantification was obtained by after-column derivatization with Cu-bicinchoninate (0.35 ml min^{-1}) at 105 ℃ in a 30 m crocheted Teflon coil of 0.3 mm inner diameter and the detection at 560 nm, which was according to Willfö et al. (2009). Data were analysed with the Dionex Chromeleon software.

Fig. 2.9. Dionex Ultimate 3000 HPLC.

2.6 Transmission electron microscopy (TEM) studies of degraded bamboo

Degraded bamboo samples from the preserving jar experiment (Chapter 2.4.1) were used for TEM. Sections were taken from the first 0.5 mm of the fungi-infected sample surface. The samples were air dried and embedded in Spurr's epoxy resin under mild vacuum according to Kleist and Schmitt (1999). Ultrathin (80-100 nm) transverse sections of the embedded specimens were cut with a diamond knife, placed on uncoated 300-mesh copper grids, and stained with potassium permanganate according to Donaldson (1992). Examination was carried out with a Philips CM 12 TEM at accelerating voltages of 40 or 60 kV to enhance contrast (Fig. 2.10).

Fig. 2.10. Philips CM12 transmission electron microscope.

2.7 Ultraviolet microspectrophotometry (UMSP) studies of degraded bamboo

Sections of degraded bamboo samples from the preserving jar experiment (Chapter 2.4.1) for UV were taken from the first 0.5 mm of the fungi-infected sample surface. The samples were fixed in glutaraldehyde/paraformaldehyde solution, dehydrated in a graded series of acetone and embedded in epoxy resin according to Spurr (1969) to stabilize the degraded cell wall structure and facilitate the cutting procedure. The embedded samples were cut into 1-µm semi-thin cross sections with an ultramicrotome Ultracut E (Reichert-Jung) with a diamond

knife. Thereafter, the sections were transferred to quartz microscope slides, thermally fixed, mounted in non-UV-absorbing glycerol, and covered with a quartz cover slip.

2.7.1 Light microscopic analysis

As preparation for the UMSP measurements, 1-µm thin sections from the preserving jar experiment (Chapter 2.4.1) were stained with toluidine blue (Gerlach 1977) to enhance the cell wall structure of the tissue. Sections were immersed in glycerol and examined with the Zeiss Ultraphot II microscope and Olympus BX 51 microscope, adapted with the CELL F (Olympus) evaluation program.

2.7.2 UV-absorbance spectra measurement

Analyses were carried out using a Zeiss UMSP 80 microspectrophotometer (Fig. 2.11). The lignin modification process within the S_2 layer was additionally investigated by photometric point-by-point measurements (spot size 1 µm²) between 240 and 400 nm wavelengths using the program LAMWIN (Zeiss). This program evaluates the UV absorbance spectra according to Lambert-Beer's law. The point measurements were automatically repeated 6 times at each spot for individual wall layers: (1) ML-F (middle lamella of fibre), (2) secondary wall of fibre, (3) ML-P (middle lamella of parenchyma cell), (4) secondary wall of parenchyma cell, (5) secondary wall of vessel. For quantitative studies, 6 spectra were taken from each cell wall layer.

Fig. 2.11. Zeiss UMSP 80 microspectrophotometer.

2.7.3 UV-scanning measurement

The Zeiss UMSP 80 microspectrophotometer equipped with a scanning stages enables the determination of image profiles at constant wavelengths using the scan program APAMOS (automatic photometric analysis of microscopic objects by scanning; Zeiss) as described by Koch and Kleist (2001). The scan program digitises square fields of the tissue with a local geometrical resolution of 0.25 x 0.25 µm and a photometrical resolution of 4096 grey scale levels, which are converted into 56 basic colours to visualise the local absorbance intensities. The scan can be depicted as a two- or three-dimensional image profile, including a statistical evaluation (histogram) of the semiquantitative lignin distribution. Ten to 12 measurements of individual wall layers attacked by fungi were carried out: (1) thick fibres in the vascular bundle, (2) thin fibres in the ground parenchyma, (3) parenchyma cells, (4) vessel-fibre area, (5) vessel-parenchyma area.

3 Results and Discussion

3.1 Identification of bamboo inhabiting fungi

3.1.1 Moulds and Basidiomycetes on bamboo culms

Many of the obtained culm sections showed spots of different size, mostly on their outer surface and only rarely inside. The colours varied due to the presence of conidia from whitish, often gray and green to blackish. Most of these spots consisted of sparse and flat, rarely woolly mycelia. These features are characteristic of moulds (Deuteromycetes). Fig. 3.1 shows some examples of stained culm sections.

Fig. 3.1. Examples of stained culm sections from Philippines.

Inoculations were done from the moulded spots on agar to obtain pure cultures for subsequent molecular identification via the DNA.

Basidiomycete-like fruiting bodies were rarely present. Some could be easily identified by macroscopic features like *Schizophyllum commune* (Fig. 3.2). From fruiting bodies, mycelium was aseptically sampled from the inner to obtain pure cultures for molecular identification and subsequent bamboo degradation tests. Several fruiting bodies were already dry, and their subculturing was unsuccessful. Due to the huge amount of samples, it was not tried to extract DNA directly from those dry fruiting bodies. Examples of culms with fruiting bodies are shown in Fig. 3.2.

Fig. 3.2. Fruiting bodies on bamboo samples.
Left and middle: *Schizophyllum commune*; right: probably *Xylaria hypoxylon* (ascomycete).

In total, 150 strains were isolated and brought to pure cultures. Of these, 76 were identified (Table 3.1).

Table 3.1. Isolated and identified fungi from infected bamboos

Country	Number of isolates	Number of identified isolates
Ethiopia	5	1
China	25	18
Costa Rica	9	1
Germany	6	5
Indonesia	5	1
Philippines	15	2
Thailand	43	19
Vietnam	42	29
Total	150	76

The most frequent isolates were found on bamboos from Asian countries, China (25), Thailand (43) and Vietnam (42). Hyde et al. (2002) summarised that the greatest diversity of fungi on bamboo is known from Asia with about 500 species, followed by South America (180), India (90) and North America (70). The high number of bamboo inhabiting fungi in Asia may be attributed to the high diversity of Asian bamboos. Forty-four bamboo genera, 60% of the world's total number, occur throughout tropical, subtropical and temperate Asia. This great diversity of plant species in an area is likely to support an equally diverse Mycota occurrence. The lower number of fungi described from non-Asian regions may also be attributed to limited surveying.

However, it should be considered that it is impossible to define the time and locality where the infection of the culms took place. Mould growth is particularly favoured by high moisture at the substrate surface (culm epidermis) and high relative air humidity (Schmidt 2006). Thus in the above cases, infections could have been happened already on the living plant. Several Deuteromycetes and Ascomycetes have been reported to colonize bamboos in the forests (Mohanan 1997). However, it is assumed that at least the most infections took place after harvest and particularly during culm storage in the moist tropical climate. A warm temperature is favourable but not absolutely necessary for mould development; they can even grow slightly above the freezing point such as on bread and jam at 4 ℃ in the refrigerator. Moulds do not like air circulation. Thus, the oversea transport in containers may have also led to infections. Altogether, those culms often show severe colonization by moulds (Fig. 2.1). Under suitable conditions on the storage place after culm arrival, further fungal infection and development are improbable. With regard to bamboo moisture content, the sensitive phase for fungal attack lies in the first eight weeks with moisture content above the fibre saturation point (Tang et al. 2012).

3.1.2 Identified fungi from bamboo by DNA sequencing

A total of 76 fungal isolates were identified via fruiting bodies and mycelial pure cultures representing 16 genera and 37 species. The most frequent isolates belong to the Deuteromycetes/Ascomycetes (67 isolates) which accounts to 88 %. The other nine 9 isolates are basidiomycetes, which account to 12 %. In comparison with previous studies, Basidiomycetes were only rarely isolated in this study. Kim et al. (2011) obtained 34 basidiomycete isolates from 127 fungal isolations.

Table 3.2 lists the identified fungi.

Table 3.2. Identified isolates from bamboo by DNA sequencing

Bamboo origin	Deuteromycetes/Ascomycetes (number of strains)	Basidiomycetes (number of strains)
Ethiopia		*Schizophyllum commune* (1)
China	*Alternaria alternata* (1) *Alternaria tenuissima* (1) *Arthrinium phaeospermum* (1) *Cladosporium cladosporioides* (2) *Dothiorella gregaria* (1) *Fusarium asiaticum* (1) *Fusarium culmorum* (1) *Fusarium zeae* (1) *Nigrospora oryzae* (4) *Penicillium commune* (1) *Penicillium chrysogenum* (1) *Penicillium tricolor* (1) *Penicillium variabile* (1) *Phoma macrostoma* (1)	
Costa Rica		*Schizophyllum commune* (1)
Germany	*Trichoderma koningiopsis* (2) *Trichoderma viride* (3)	
Indonesia		*Cyathus stercoreus* (1)
Philippines	*Penicillium citrinum* (1) *Penicillium sumatraense* (1)	
Thailand	*Aspergillus nomius* (1) *Aspergillus repens* (1) *Botryosphaeria subglobosa* (1) *Cladosporium cladosporioides* (2) *Epicoccum nigrum* (2) *Penicillium brevicompactum* (1) *Penicillium citrinum* (2) *Penicillium pinophilum* (1) *Trichoderma atroviride* (1) *Trichoderma koningiopsis* (1)	*Schizophyllum commune* (6)
Vietnam	*Apiospora montagnei(2)* *Arthrinium phaeospermum* (1) *Arthrinium sacchari* (3) *Aspergillus flavus* (5) *Aspergillus niger* (2) *Botryosphaeria subglobosa* (5) *Epicoccum nigrum* (4) *Penicillium bialowiezense* (1) *Penicillium biourgeianum* (1) *Penicillium brevicompactum* (2) *Penicillium expansum* (1) *Penicillium islandicum* (1) *Pestalotiopsis microspora* (1)	
Total number of identified isolates	67	9

Among the 67 Deuteromycetes/Ascomycetes, 35 species representing 14 genera have been identified. The most frequent genus was *Penicillium* (24 %, 16 isolates), followed by *Aspergillus* (13 %, 9 isolates). Some pure cultures are shown in Fig. 3.3.

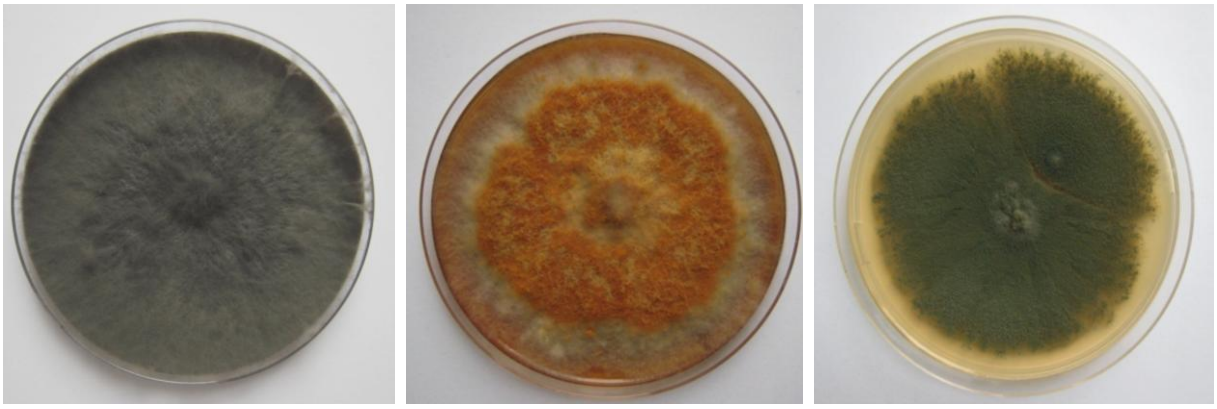

Fig. 3.3. Mould isolates from bamboo.
Left: *Botryosphaeria subglobosa* from Vietnam; middle: *Epicoccum nigrum* from Thailand; right: *Penicillium commune* from China.

Among the nine basidiomycete isolates (Table 3.2), *Schizophyllum commune* was isolated eight times and *Cyathus stercoreus* once. *Schizophyllum commune* can be easily identified by its fruiting body. The fungus is common on bamboo (Fig. 1.2) and wood. As a white-rot basidiomycete, *S. commune* is capable of utilizing all structural components of the lignified cell wall. *Cyathus stercoreus* is a new record for bamboo. Neither soft-rot nor brown-rot fungi were found in this investigation, as it was already reported by Kim et al. (2001).

The reason of not finding soft-rot fungi can only be assumed. These fungi preferentially attack lignocelluloses of high moisture content, particularly in substrates with soil or water contact (Liese 1959b, Schmidt 2006); the investigated culms did not derive from those conditions. However, soft-rot decay was found in the stakes from the field test (see chapter 3.4.5).

An explanation of not detecting brown-rot fungi in bamboo may be that this fungal group prefers softwoods and is rarely found on hardwoods, like *Daedalea quercina* on oak wood, whereas white-rot species prefer hardwoods. Brown-rot fungi consume the carbohydrates cellulose and hemicelluloses of the lignified cell wall. To my knowledge, differences of the cellulose characteristics between soft- and hardwoods are not described. However, the main hemicellulose of bamboo is of the xylan type (see chapter 3.5) as is also the case for hardwoods, whereas softwoods contain mainly mannans (Schmidt 2006). Therefore, the

hemicellulose xylan of bamboo (Vena et al. 2013: 21.6 %, Table 3.15: 19 to 27 %) may have favour white-rot species and inhibit brown-rot fungi.

3.2 Identification of some *Penicillium* and *Aspergillus* isolates

Some mould genera such as *Aspergillus* and *Penicillium* contain very closely related species of which some also have various synonyms. This may lead to mis-identifications. Therefore, further morphological and molecular experiments were done.

3.2.1 Morphological characterization of *Penicillium* and *Aspergillus* isolates

Some *Penicillium* and *Aspergillus* isolates from bamboos were inoculated on different agar substrates. Fig. 3.4 shows as an example the colony characteristics of the *Penicillium albobiverticillium* after one week incubation at 25 ℃ or 37 ℃.

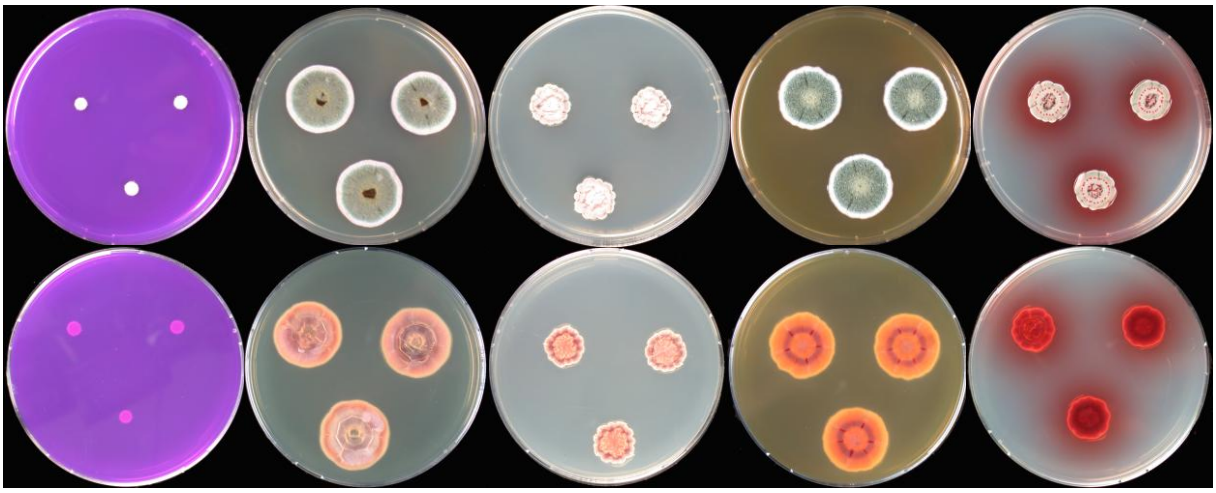

Fig. 3.4. Colony appearance of the *Penicillium albobiverticillium* on various agars.
From left: creatine agar at 25 ℃; Czapek yeast extract agar (CYA) at 25 ℃; CYA at 30 ℃; malt extract agar at 25 ℃; yeast extract sucrose agar at 25 ℃.

Colony characteristics were recorded after 7 d of incubation. Fig. 3.5 shows *Aspergillus flavus* growth on different media.

Fig. 3.5. Colony appearance of an *Aspergillus flavus* on various agars.
From left: Czapek yeast extract agar (CYA) at 25 ℃, CYA at 37 ℃, malt extract agar at 25 ℃

3.2.2 Molecular identification of *Penicillium* and *Aspergillus* isolates

Particularly both genera *Penicillium* and *Aspergillus* contain many closely related and morphologically similar species, which are hardly to be differentiated by the rDNA-ITS sequence. Furthermore, the international DNA databases are man-made and contain mistakes, such as misidentifications, e.g. if a deposited sequence was related to a wrongly named fungus. Therefore, the sequences of ß-tubulin and calmodulin were additionally used for identification. DNA extraction, PCR, sequencing and BLAST-identification are described in Chapter 2.1 and 2.2.

Table 3.2 shows the some results of *Penicillium* and *Aspergillus* isolates.

Table 3.2. Comparison of identifications of *Penicillium* and *Aspergillus* isolates obtained by their rDNA-ITS, ß-tubulin and calmodulin sequences

Fungal strain coding	ITS		ß-Tubulin		Calmodulin	
	Species	Max. identity (%)	Species	Max. identity (%)	Species	Max. identity (%)
D54	*Aspergillus nomius*	100	*Aspergillus nomius*	99	*Aspergillus nomius*	99
			Aspergillus zhaoqingensis	99	*Aspergillus zhaoqingensis*	99
D23-1	*Penicillium biourgeianum*	100	*Penicillium biourgeianum*	100	*Penicillium biourgeianum*	98
D19-2	*Penicillium brevicompactum*	100	*Penicillium brevicompactum*	98	*Penicillium brevicompactum*	99
D36	*Penicillium* sp.	100	*Penicillium phialosporum*	90	*Penicillium variabile*	82
D80-2	*Penicillium commune*	100	*Penicillium commune*		*Penicillium commune*	99
D46	*Penicillium citrinum*	100	*Penicillium citrinum*	100	*Penicillium citrinum*	100
D9-1	*Penicillium expansum*	100	*Penicillium expansum*	99	*Penicillium expansum*	97
D57	*Penicillium verruculosum*	100	*Penicillium* sp.	87	*Penicillium aculeatum*	87
	Penicillium aculeatum	98			*Penicillium pinophilum*	85
D79-1	*Penicillium expansum*	100	*Penicillium expansum*	100	*Penicillium expansum*	97
D65-1	*Penicillium polonicum*	100	*Penicillium polonicum*	100	*Penicillium commune*	92
					Penicillium thymicola	92

Some isolates like strains D23-1 and D19-2 revealed identical names with all three DNA sequences, other isolates (like D54) showed quite different naming. However, it has to be considered that some isolates are obviously not represented in the databases by all three sequences like *Penicillium verruculosum* D57.

Altogether, several moulds were isolated from bamboo culms and could be identified by molecular techniques down to the species level. Some moulds are ubiquitous. Detection of *Fusarium asiaticum* and *Penicillium sumatraense* may be a hint that bamboo infection has occurred in their home country.

The following chapters describe blue-stain and decay experiments with bamboo culm samples. The fungi used are own identifications from bamboos or derive from the laboratory strain collection.

3.3 Blue-stain test

3.3.1 Discolouration of samples by blue-stain fungi

Most of the bamboo samples were already overgrown by blue-stain fungi after two weeks of cultivation and discoloured after four weeks to gray-blue. Bamboo samples inoculated with *Aureobasidium pullulans* are shown in Fig. 3.6.

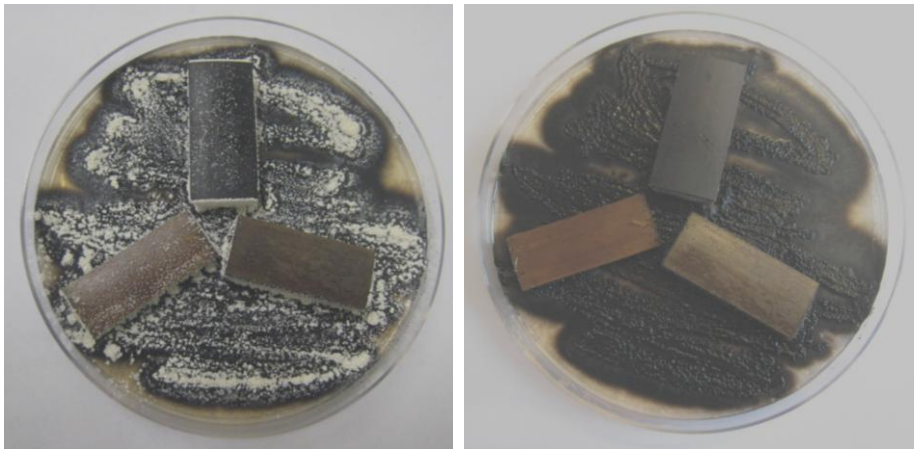

Fig. 3.6. Bamboo samples inoculated with *Aureobasidium pullulans* 87.
Left: 4 weeks; right: 20 weeks.

Fig. 3.7 shows the light control samples of the three bamboo species tested and the discoloured samples after 20 weeks incubation. There were differences both in colour between the bamboos and the fungi, respectively. Because the picture shows the dried samples, the colours were lighter than directly after incubation.

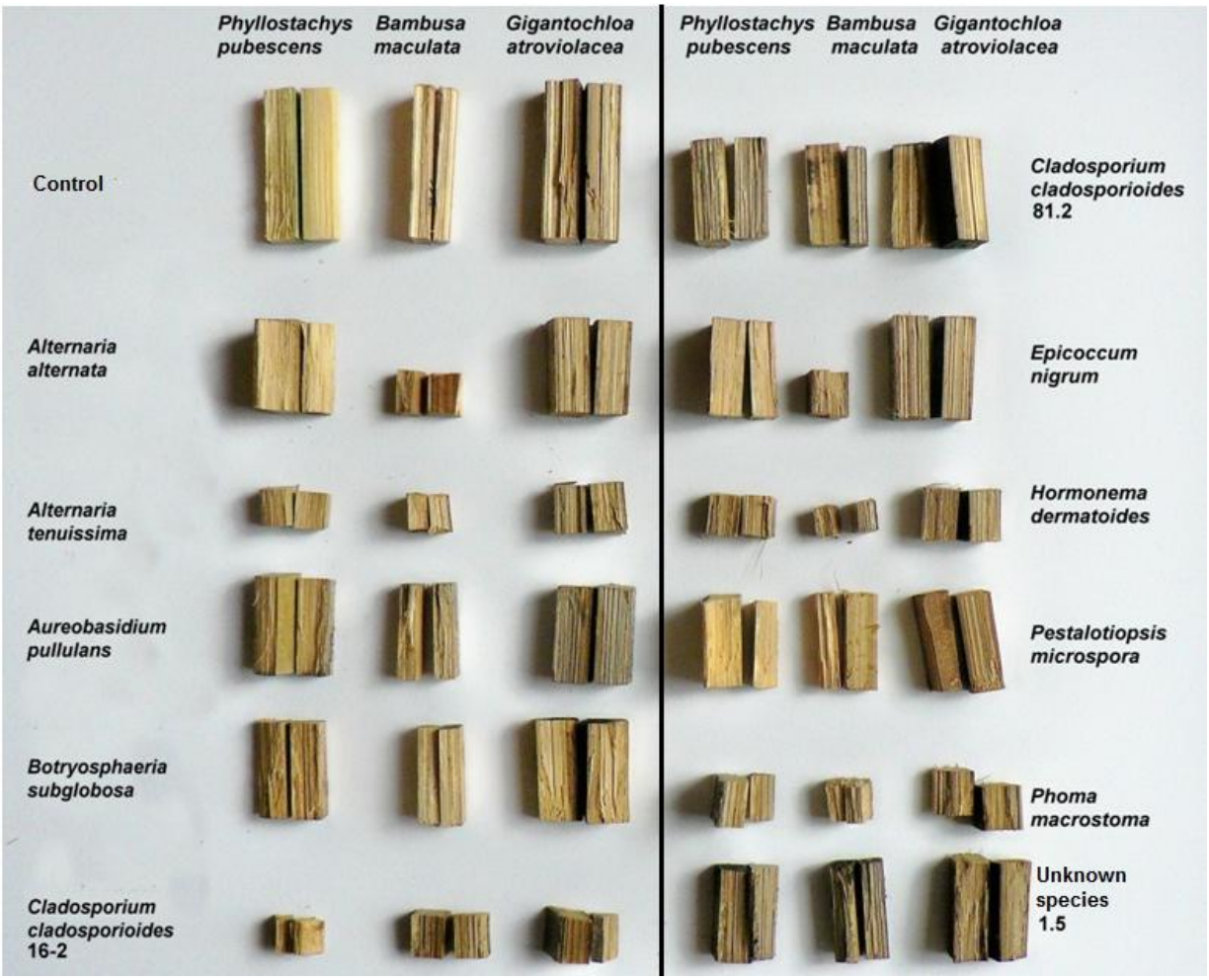

Fig. 3.7. Dry bamboo samples after 20 weeks of incubation with blue-stain fungi.

3.3.2 Microscopic characterises of blue-stain in bamboo

The colonization of the tissue (vessels and parenchyma) by blue-stain fungi was observed by light microscopy after 4 and 20 weeks incubation. Fig. 3.8 shows some examples. The samples were colonized by the typically thick, brown hyphae and chlamydospores of blue-stain fungi. In some cases, a transpressorium, a thin penetration hypha was found. The transpressorium is the only fungal `organ´by which blue-stain fungi damage lignified cell walls (Liese and Schmid 1966). It is still unknown whether the penetration of the cell wall is by mechanical and/or enzymatic action.

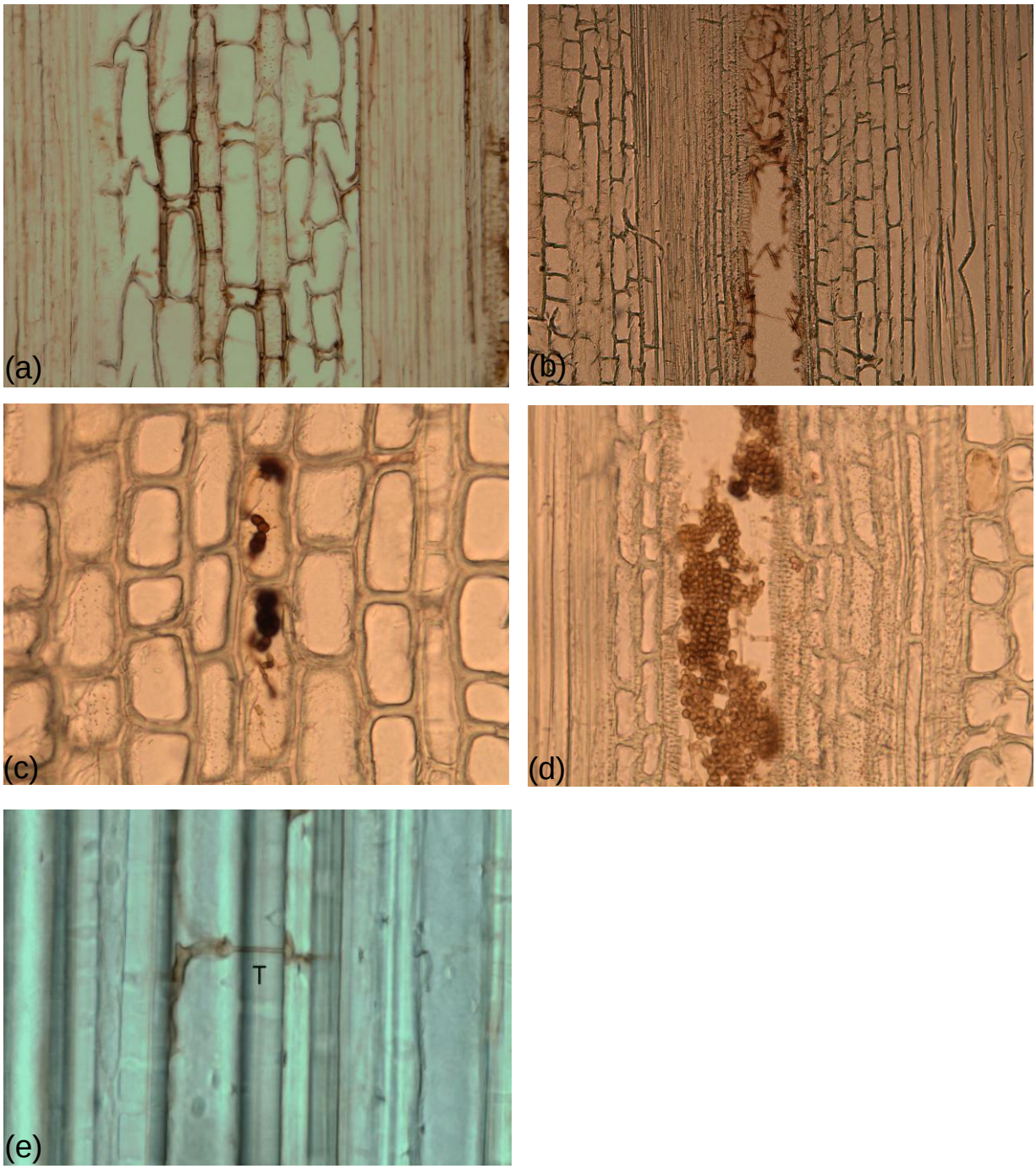

Fig. 3.8. Hyphae, chlamydospores and transpressorium of blue-stain fungi in bamboo.
(a): hyphae of the non-identified isolate 1.5 in parenchyma cells of *Gigantochloa atroviolacea* (200×); (b): hyphae of *Hormonema dematioides* in a vessel of *G. atroviolacea* (100×); (c): chlamydospores of *Phoma macrostoma* D64-1 in parenchyma cells of *Phyllostachys pubescens* (200×); (d): chlamydospores of *P. macrostoma* D64-1 in a vessel of *P. pubescens* (200×); (e): transpressorium (T) of *Alternaria alternata* in *Bambusa maculata* (600×).

3.3.3 Development of hyphae and chlamydospores in bamboo

Tables 3.4 and 3.5 summarize the abundance of hyphae in vessels and parenchyma cells.

Table 3.4. Abundance of hyphae in vessels

Species and isolate	*Bambusa maculata*		*Gigantochloa atroviolacea*		*Phyllostachys pubescens*	
	4 weeks	20 weeks	4 weeks	4 weeks	20 weeks	4 weeks
Alternaria alternata D84-2	1	3	3	4	2	4
Alternaria tenuissima D82-3-2	0	1	2	1	1	1
Aureobasidium pullulans 87	2	3	2	3	2	4
Botryosphaeria subglobosa D26	1	1	1	1	2	4
Cladosporium cladosporioides D16-2	1	1	4	4	2	4
Cladosporium cladosporioides D81-2	1	2	4	4	4	4
Epicoccum nigrum D20	0	0	1	2	1	1
Hormonema dematioides 85	2	2	1	4	1	3
Pestalotiopsis microspora D24	0	0	2	2	0	1
Phoma macrostoma D64-1	1	2	1	3	1	4
Unidentified isolate 1.5	1	1	1	3	1	4

Table 3.5. Abundance of hyphae in parenchyma cells

Species and isolates	*Bambusa maculata*		*Gigantochloa atroviolacea*		*Phyllostachys pubescens*	
	4 weeks	20 weeks	4 weeks	20 weeks	4 weeks	20 weeks
Alternaria alternata D84-2	2	2	2	4	1	1
Alternaria tenuissima D82-3-2	0	0	1	1	1	1
Aureobasidium pullulans 87	2	2	1	3	1	1
Botryosphaeria subglobosa D26	1	1	2	2	1	3
Cladosporium cladosporioides D16-2	1	1	1	3	2	3
Cladosporium cladosporioides D81-2	1	2	1	1	0	1
Epicoccum nigrum D20	0	0	1	2	0	0
Hormonema dematioides 85	2	2	1	2	1	1
Pestalotiopsis microspora D24	1	1	2	2	0	1
Phoma macrostoma D64-1	1	2	1	1	1	1
Unidentified isolate 1.5	1	1	1	4	1	2

In general, the different bamboo species were colonized to a different degree by the blue-stain fungi. Most samples were already colonized within 4 weeks. Often, colonization of the tissue increased with incubation time. The fast colonization of the bamboo tissue by blue-stain fungi indicates that the culms are highly endangered for blue-stain within the first 2 months after harvest when the moisture content in the culms is above fibre saturation.

Blue-stain fungi form chlamydospores with which can survive unsuitable environments and germinate again to hyphae under suitable conditions. Chlamydospores were not observed for all isolates, but were found in all three bamboos. Tables 3.6 and 3.7 summarize their frequencies in vessels and parenchyma cells.

Table 3.6 Abundance of chlamydospores in vessels

Species and isolates	*Bambusa maculata*		*Gigantochloa atroviolacea*		*Phyllostachys pubescens*	
	4 weeks	20 weeks	4 weeks	4 weeks	20 weeks	4 weeks
Alternaria alternata D84-2	1	3	3	4	2	4
Alternaria tenuissima D82-3-2	0	0	2	1	0	0
Aureobasidium pullulans 87	2	3	2	3	2	4
Botryosphaeria subglobosa D26	1	1	1	1	0	4
Cladosporium cladosporioides D16-2	0	0	0	0	0	0
Cladosporium cladosporioides D81-2	1	2	4	4	4	4
Epicoccum nigrum D20	0	0	0	0	0	0
Hormonema dematioides 85	2	2	1	4	1	3
Pestalotiopsis microspora D24	0	0	2	2	0	0
Phoma macrostoma D64-1	1	2	1	3	1	4
Unidentified isolate 1.5	0	0	0	0	0	0

Table 3.7 Abundance of chlamydospores in parenchyma cells

Species and isolates	*Bambusa maculata*		*Gigantochloa atroviolacea*		*Phyllostachys pubescens*	
	4 weeks	20 weeks	4 weeks	4 weeks	20 weeks	4 weeks
Alternaria alternata D84-2	2	2	2	4	1	1
Alternaria tenuissima D82-3-2	0	0	1	1	0	0
Aureobasidium pullulans 87	2	2	1	3	1	1
Botryosphaeria subglobosa D26	1	1	2	2	0	3
Cladosporium cladosporioides D16-2	0	0	0	0	0	0
Cladosporium cladosporioides D81-2	1	2	1	1	0	1
Epicoccum nigrum D20	0	0	0	0	0	0
Hormonema dematioides 85	2	2	1	2	1	1
Pestalotiopsis microspora D24	0	0	2	2	0	0
Phoma macrostoma D64-1	1	2	1	1	1	1
Unidentified isolate 1.5	0	0	0	0	0	0

3.4 Degradation tests

For information on the susceptibility of bamboo culms to rot fungi, degradation tests were performed with various experimental set-ups. Basidiomycetes derived from the laboratory strain collection or are our own isolations were used.

3.4.1 Preserving jar test

Fig. 3.9 shows the results for the brown-rot fungi *Coniophora puteana* and *Gloeophyllum trabeum*.

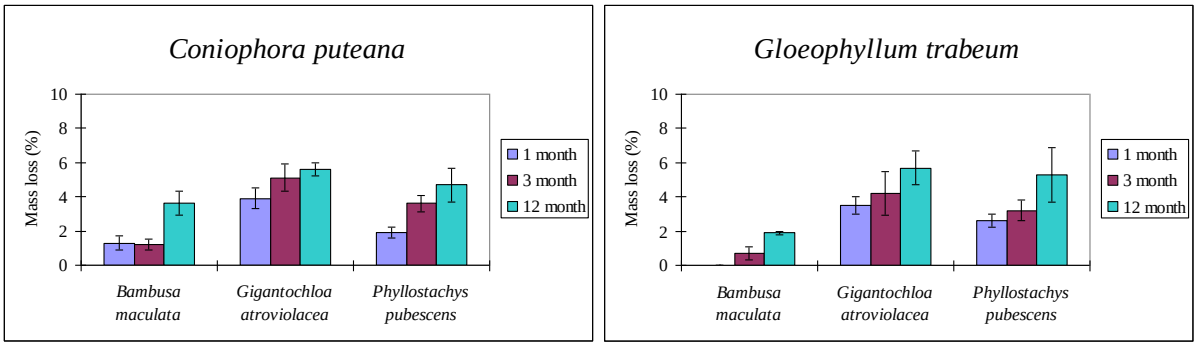

Fig. 3.9. Mass loss caused by brown-rot fungi in preserving jars.

Both brown-rot fungi were relatively inactive within 12 months (max. 5.7 % ML). This contrasts to observations by Lee et al. (2006) who reported a 25 % ML by *G. trabeum* in *Phyllostachys pubescens* and by Ma et al. (2010) who found a 35 % ML in *P. edulis*. However, strain variation within a species should be considered (Schmidt 2006). Both author groups used other isolates and also other incubation conditions.

Fig. 3.10 shows the results for the soft-rot fungi *Paecilomyces variotii* and *Chaetomium globosum*.

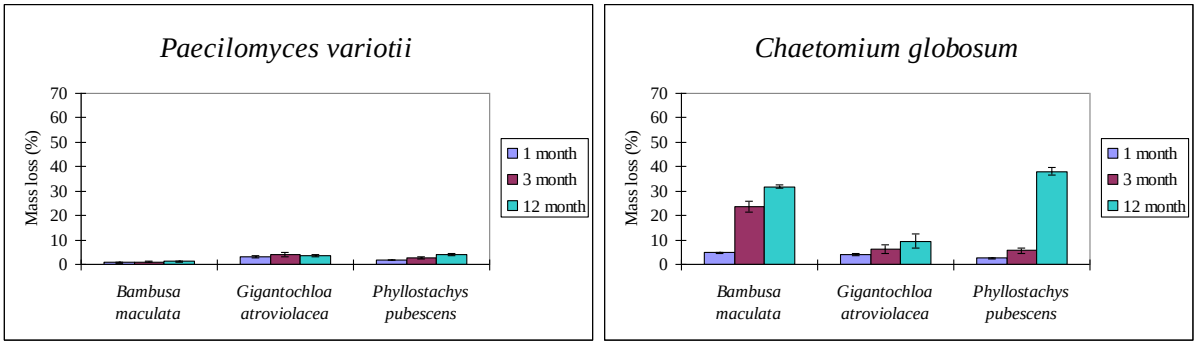

Fig. 3.10. Mass loss caused by soft-rot fungi in preserving jars.

Of the soft-rot fungi, *Paecilomyces variotii* was less aggressive, whereas *Chaetomium globosum* produced severe degradation with *P. pubescens* (max. 38.0 %), which also occurred in other experiments of this study. A Japanese isolate of *Ch. globosum* caused less decay (Suprapti 2010).

Fig. 3.11 shows the results for the white-rot fungi *Schizophyllum commune*, *Cyathus stercoreus*, *Pleurotus ostreatu* and *Trametes versicolor*.

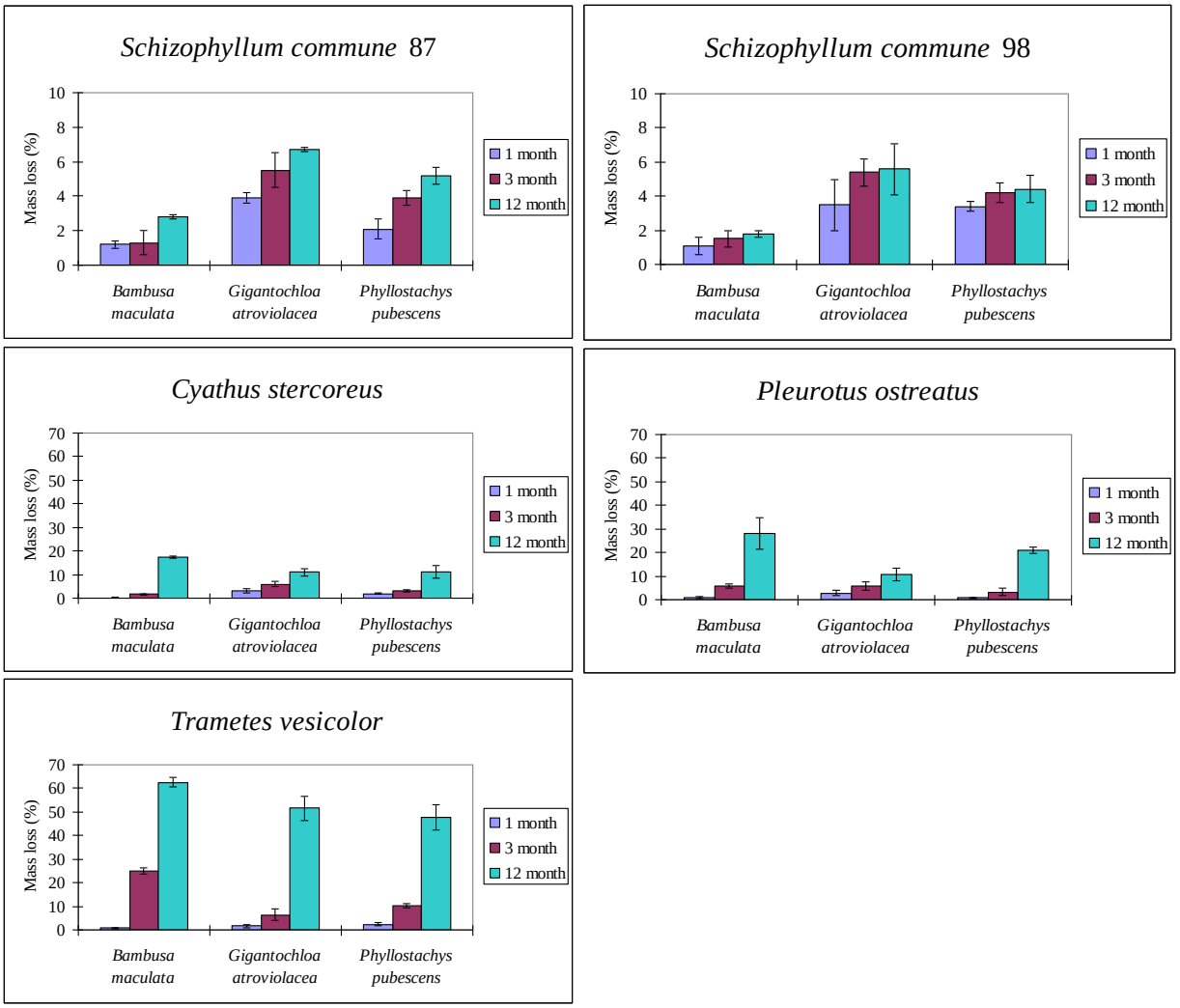

Fig. 3.11. Mass loss caused by white-rot fungi in preserving jars.

Among the white rot fungi (Fig. 3.11), *Schizophyllum commune* caused the least decay (max. 6.7 %), *Cyathus stercoreus* and *Pleurotus ostreatus* medium degradation (max. 28.2 %) and *Trametes versicolor* the highest decay (max. 62.5 %).

Cyathus stercoreus (Nidulariaceae) (Fig. 3.12) is found in nature on severely rotten wood debris and on manured or burned soils (Breitenbach and Kränzlin 1986). It was not yet reported for bamboo and showed low activity (Fig. 3.11).

Fig. 3.12. Fruiting bodies of *Cyathus stercoreus* on wood chips.
(photo: www.mushroomexpert.com).

Zhang et al. (2007) investigated 34 white-rot fungi and observed up to 15 % ML in *P. pubescens*; *Pleurotus ostreatus* caused 5.2 % degradation and *Trametes versicolor* 13.6 % decay. The white-rot fungus *Lentinula edodes* produced a 13 % ML in *P. pubescens* (Kim et al. 2008). Abdurachim (1964) reported 15 % ML by *S. commune*. A low decay rate by *S. commune* was found by Suprapti (2010) and Kim et al. (2011) and was also reported for wood samples (Schmidt and Liese 1978). However, this species is commonly found on bamboo culms during storage and use (Fig. 1.2; Liese 1985, Mohanan 1997, Liese and Kumar 2003). Kleist et al. (2002) showed that *S. commune* was the most successful coloniser among some fungi as it could penetrate bamboo via outer and inner culm cell walls, through cross section planes as well as through nodal ridges and longitudinally through nodia.

When comparing the results with the literature, the possibility of chemical pretreatment of bamboo against fungi should be kept in mind. For example, boron is often used before shipment to Europe and would severely affect subsequent decay experiments. Our samples were boron-free as proven by the curcuma-test. Furthermore, the results of all experiments on fungal activity strongly depend on the specific strain of a species. Strain variation is common among fungi, occurs also in *S. commune* (Schmidt and Liese 1980). Fig. 3.11, however, shows similar results for the two *S. commune* isolates.

3.4.2 Fungus cellar test

To imitate natural conditions, the less active fungi *S. commune* and *C. puteana* were tested in the `Fungus cellar´. With this test set-up (Fig. 2.4), larger samples are incubated on unsterile soil in large metal tubes (Gersonde and Becker 1958). Samples were either directly placed on the soil or on wooden supports. Table 3.8 summarizes the obtained results.

Table 3.8. Mass loss (ML, %) and final moisture content (MC, %) of bamboo samples after one year in the Fungus cellar test

Species	Tub	Soil	*Coniophora puteana* 167		*Schizophyllum commune* 87	
		contact	ML (%)	MC (%)	ML (%)	MC (%)
Arundinaria	1	+	15.5	187	15.3	148
amabilis	1	-	38.6	41	10.8	31
	2	-	43.4	57	8.7	68
	2	-	39.0	56	7.1	40
Bambusa	1	+	9.9	159	11.0	174
maculata	1	-	20.4	39	5.1	28
	2	-	38.3	48	5.0	33
	2	-	34.3	52	3.6	37
Dendrocalamus	1	+	5.4	104	5.1	90
asper	1	-	29.3	42	4.3	28
	2	-	26.9	45	3.8	32
	2	-	28.5	50	4.3	31
Gigantochloa	1	+	6.1	95	6.1	95
atroviolacea	1	-	18.7	34	4.7	25
	2	-	41.6	58	3.0	38
	2	-	42.9	57	5.0	43
Phyllostachys	1	+	9.7	112	16.4	126
nigra	1	-	32.6	49	9.1	32
	2	-	39.7	65	6.6	40
	2	-	40.7	56	6.4	52
Phyllostachys	1	+	35.3	103	19.7	182
nigra `Boryana´	1	-	38.8	54	6.7	26
	2	-	37.9	60	5.7	40
	2	-	38.8	46	5.1	35
Phyllostachys	1	+	6.3	61	6.3	63
pubescens	1	-	5.4	32	5.4	24
	2	-	38.3	42	5.7	31
	2	-	31.2	43	6.3	36

Due to the not absolutely identical conditions in the two tubs, Table 3.8 shows the data of all samples. In contrast to results with small samples in the jar test, bamboo samples in the Fungus cellar were considerably more degraded by *C. puteana* (max. 43 %) and *S. commune* (max 20 %). Obviously, the moisture conditions in the Fungus cellar test influenced the activity of both fungi, whereby the white-rot fungus *S. commune* differed considerably from the brown-rot species *C. puteana*. *Schizophyllum commune* decayed samples with soil contact and thus with high water content (90-182 % u) more than samples without soil contact. In contrast, *C. puteana* produced a higher ML in samples located on wood supports and with lower water content (34-65 % u). In view of practice, bamboo samples in the moisture range from 24 to 182 % were attacked by basidiomycetes. However, due to the unsterile soil in the Fungus cellar, samples became contaminated by moulds, which are capable of excreted growth promoting substances for *S. commune*. Components from the soil or vitamins from soil bacteria may have also affected the fungus (Schmidt et al. 2011).

3.4.3 Kolle flask test

3.4.3.1 Growth of fungi and final moisture content

The development of hyphal growth was measured by recording the density of hyphal mats and the percentage of covering the sample surface (Table 3.9).

After initial differences between the fungi within the first two weeks, most bamboo species were covered by mycelium after 16 weeks between two thirds of the surface and total coverage. An exception with low surface growth occurred on *Guadua angustifolia* samples. In general, coverage correlated with hyphal density. The density of mycelium is a specific feature for many fungi (Stalpers 1978). However, it does not relate to actual fungal activity within the substrate (Schmidt 2006). *Gloeophyllum trabeum* 183 did not show any hyphae on the surface of samples from *Bambusa maculata, Dendrocalamus asper* and *G. angustifolia*. However, this strain produced some degradation (Fig. 3.12), obviously not by surface growth but by substrate mycelium. The final moisture content of the samples was from 39 to 101%, which is a suitable range for fungal degradation.

Table 3.9. Mycelium growth on bamboos after 16 weeks of incubation

	Bambusa maculata		*Dendrocalamus asper*		*Gigantochloa atroviolacea*		*Guadua angustifolia*		*Phyllostachys pubescens* China		*Phyllostachys pubescens* Germany	
	HD	HC	HD	HC	HD	HC	HD	HC	HD	HC	HD	HC
Coniophora puteana 15	2	4	1	3	2	4	1	0	2	4	3	4
Coniophora puteana 167	2	4	1	4	2	4	1	1	2	4	2	4
Coniophora puteana 159	2	4	2	4	2	4	2	2	2	4	2	4
Coniophora puteana 247	2	4	2	4	2	4	0	0	2	4	2	4
Gloeophyllum trabeum 183	0	0	0	0	1	4	0	0	1	4	1	4
Gloeophyllum trabeum 259	1	2	1	4	1	4	0	0	1	3	1	4
Trametes versicolor	1	4	2	4	2	4	1	2	3	4	2	4
Schizophyllum commune	2	4	1	4	2	4	0	0	2	4	2	4
Chaetomium globosum	2	4	2	4	2	4	1	2	2	4	2	4
Paecilomyces variotii	2	4	2	4	2	4	0	0	2	4	2	4

HD = Hyphal density: 0 = no growth; 1 = sparse mycelium; 2 = normal mycelium; 3 = thick mycelium.
HC = Hyphal coverage: 0 = no coverage; 1 = 1-33 % coverage; 2 = 34-66 % coverage; 3 = 67 - 99 % coverage; 4 = total coverage.

3.4.3.2 Durability of bamboo against fungi

All five bamboo species were rather resistant to degradation by the various strains of the brown-rot fungi *C. puteana* and *G. trabeum* (Fig. 3.13) without significant variation among the strains. Both fungi comprised the test strains to be used in EN 113 (1996) for wood samples.

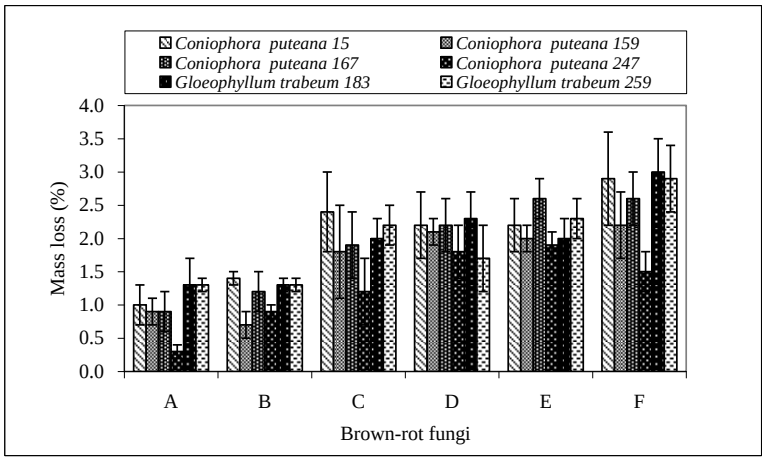

Fig. 3.13. Mass loss of bamboos caused by brown-rot fungi after 16 weeks incubation.
Error bars represent means ± standard deviation.
A: *Bambusa maculata*; B: *Dendrocalamus asper*; C: *Gigantochloa atroviolacea*; D: *Guadua angustifolia*; E: *Phyllostachys pubescens* China; F: *Phyllostachys pubescens* Germany.

Among the four *C. puteana* strains, maximum ML to bamboo was 2.9 %. According to EN 113, *C. puteana* strain Ebw. 15 shall produce about 20% mass loss in wood and wood products. The two *G. trabeum* strains also did not reveal significant ML in bamboo degradation, regardless of the species.

Mass losses of the wood controls by *C. puteana* and *G. trabeum* isolates ranged from 19.7 to 62.7 %, which shows that the conditions of the test set-up should have been also suitable for the bamboo samples.

The low activity of brown-rot fungi against bamboo correlates to the results obtained in preserving jars (Fig. 3.9) where a maximum 5.7 % ML was obtained by both brown-rot fungi on the same bamboo species. However, samples of *Melocanna bambusoides* after 6 month incubation in Kolle flasks had shown up to 13.7 % decay by *C. puteana* Ebw. 15 (Schmidt et al. 2011). To prove these earlier results, this degradation test according to EN 350 and EN 113 considered sample sterilization by gamma radiation. However, the possible influence of the sterilization technique can be only assumed. The highest ML of 3 % by *G. trabeum* is lower than the one in preserving jars. However, both results contrast to Lee et al. (2006), who found 25 % degradation by *G. trabeum* in *P. pubescens*. The reason for this discrepancy is unknown.

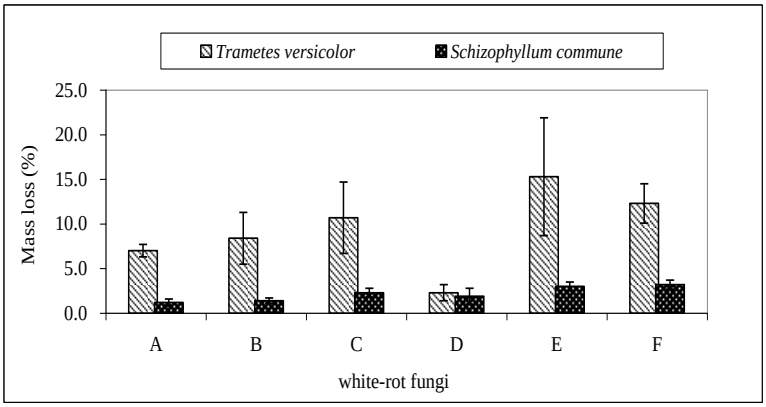

Fig. 3.14. Mass losses of bamboos caused by white-rot fungi after 16 weeks incubation. Error bars represent means ± standard deviation.
A: *Bambusa maculata*; B: *Dendrocalamus asper*; C: *Gigantochloa atroviolacea*; D: *Guadua angustifolia*; E: *Phyllostachys pubescens* China; F: *Phyllostachys pubescens* Germany.

Among the two white-rot fungi, *Trametes versicolor* showed the greatest ML with 15.3 % for *Phyllostachys pubescens* from China, followed by *P. pubescens* from Germany (12.3 %) and *G. atroviolacea* (10.4 %). *Guadua angustifolia* was very resistant (2.3 %). Degradation of the *Fagus sylvatica* control was 26.7 %.

Schizophyllum commune behaved rather inactively with maximum ML of only 3.2 %. Schmidt et al. (2011) obtained with *S. commune* maximum up to 9.1 % degradation in 6 months in Kolle flasks. A maximum of 5.6 % ML occurred during 1 year in preserving jars (Fig. 3.11). Abdurachim (1964) reported 15 % ML by *S. commune*. Low decay rates by *S. commune* were found by Suprapti (2010) and Kim et al. (2011). The beech wood control in this study showed also negligible decay (0.8 %), which corresponds to previous results (Schmidt and Liese 1978).

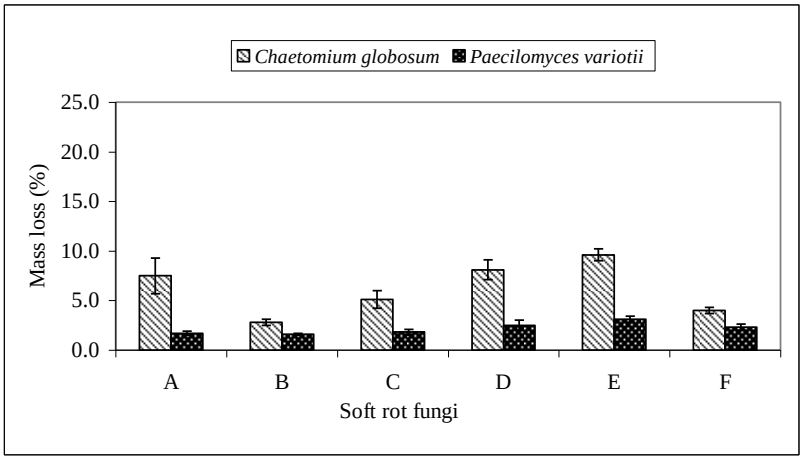

Fig. 3.15. Mass losses of bamboos caused by soft-rot fungi after 16 weeks incubation.
Error bars represent means ± standard deviation.
A: *Bambusa maculata*; B: *Dendrocalamus asper*; C: *Gigantochloa atroviolacea*; D: *Guadua angustifolia*; E: *Phyllostachys pubescens* China; F: *Phyllostachys pubescens* Germany.

The two soft-rot fungi, *Ch. globosum* and *Paecilomyces variotii* revealed significant differences with regard to bamboo decay, with higher ML by *Ch. globosum* than by *P. variotii*. Most ML was caused by *Ch. globosum* to *P. pubescens* from China (9.6%), followed by *G. angustifolia* (8.1 %) and *B. maculata* (7.5 %). Schmidt et al. (2011) reported up to 52.7 % decay by *Ch. globosum* after 6 months in Kolle flasks. In preserving jars maximum 38 % ML were measured after 1 year. Surprapti (2010) reported 8.0 % ML. Kim et al. (2011) obtained up to 17.9 % decay for other soft-rot fungi. Maximum ML by *P. variotii* was only 3.1 %. The same strain of the species had shown maximum 3.9 % degradation in preserving jars (Fig. 3.10), but another strain up to 19.7 % decay in Kolle flasks (Schmidt et al. 2011). The wood controls in our experiment revealed rather low ML around 1 % which explains that soft-rot degradation of wood samples should be tested by the specific standard EN 807 (2001).

3.4.3.3 Classification of bamboos according durability

The bamboos were grouped into 5 durability classes as it is done for wood species. Table 3.10 shows the durability classification of all bamboo/fungus combinations deriving from Figs. 3.13 to 3.15.

Table 3.10. The durability classification of bamboo

	Bambusa maculata	*Dendrocalamus asper*	*Gigantochloa atroviolacea*	*Guadua angustifolia*	*Phyllostachys pubescens* China	*Phyllostachys pubescens* Germany
Coniophora puteana 15	II	II	II	II	II	II
Coniophora puteana 167	II	II	II	II	II	II
Coniophora puteana 159	II	II	II	II	II	II
Coniophora puteana 247	II	II	II	II	II	II
Gloeophyllum trabeum 183	II	II	II	II	II	II
Gloeophyllum trabeum 259	II	II	II	II	II	II
Trametes versicolor	III	III	IV	II	IV	IV
Schizophyllum commune	II	II	II	II	II	II
Chaetomium globosum	III	II	III	III	III	II
Paecilomyces variotii	II	II	II	II	II	II

II = durable (ML < 5%), III = moderately durable (5 – 10 %), IV = slight durable (10 - 30 %)

There were considerable differences. The resistance of the five bamboo species to the brown-rot fungi *C. puteana* and *G. trabeum*, the white-rot fungus *S. commune* and the soft-rot species *P. variotii* would group them into class II (durable). The soft-rot fungus *Ch. globosum* attacked the bamboos according to classes II and III (moderately durable). Considering white-rot decay by *T. versicolor*, the five bamboo species varied between classes II and IV (slightly durable).

Because the investigated samples derived from commercial use, the details on the culm sections tested are not known. Therefore, the above results should be considered with some restrictions. The susceptibility of bamboo to fungi is influenced by various factors (Schmidt et al. 2011). Samples from young culms decayed more than older ones. Specimens from the culm top were more vulnerable to decay than those from the bottom. The cutting season influences resistance because starch and protein content vary during the year.

Furthermore, durability classifications are not only influenced by the used fungus and special fungus strain, but also by the specific test laboratory. For example, a comparative durability test among different European laboratories revealed discrepant results for several soft- and hardwoods and their corresponding test strains, even though all institutions involved used the EN standards (Brischke et al. 2013).

3.4.4 Vermiculite test

To measure the possible influence of moisture content and additional nutrients on the activity of *C. puteana* and *S. commune*, vermiculite with different amounts of water or malt extract solutions was used as support for bamboo samples. Fig. 3.16 shows examples of the vermiculite incubations and Table 3.11 summarizes the results.

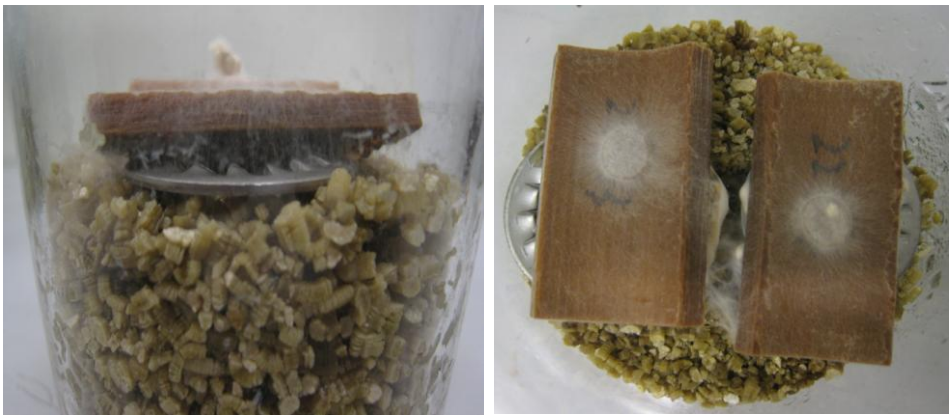

Fig. 3.16. Growth of *Schizophyllum commune* in preserving jars with vermiculite and mycelium on *Phyllostachys pubescens* samples. Left: side view; right: top view.

Table 3.11. Fungal growth on bamboo and wood samples in preserving jars with vermiculite

Bamboo	Fungus	Liquid	Liquid (ml) per preserving jar									
			80		100		120		140		160	
			D	H	D	H	D	H	D	H	D	H
Gigantochloa atroviolacea	*Coniophora puteana*	tap water	1	4	1	4	1	4	1	4	1	4
		malt extract	2	4	2	4	2	4	2	4	2	4
	Schizophyllum commune	tap water	1	4	1	4	1	4	1	4	1	4
		malt extract	2	4	2	4	2	4	2	4	2	4
Phyllostachys pubescens	*Coniophora puteana*	tap water	1	4	1	4	1	4	1	4	1	4
		malt extract	2	4	2	4	2	4	2	4	2	4
	Schizophyllum commune	tap water	1	4	1	4	1	4	1	4	1	4
		malt extract	2	4	2	4	2	4	2	4	2	4
Fagus sylvatica	*Schizophyllum commune*	tap water	1	4	1	4	1	4	1	4	1	4
		malt extract	2	4	2	4	2	4	2	4	2	4
Pinus sylvestris	*Coniophora puteana*	tap water	1	4	1	4	1	4	1	4	1	4
		malt extract	3	4	3	4	3	4	2	4	2	4

D = density of hyphae; H = hyphal coverage of sample.

All bamboo and wood samples were totally covered by mycelium after 32 weeks. Hyphal density with tap water in vermiculite as a moisture reservoir was less than with malt extract. However, the density of mycelium is not necessarily related to mass loss in test specimens.

Fig. 3.17 shows the mass loss produced by *S. commune*.

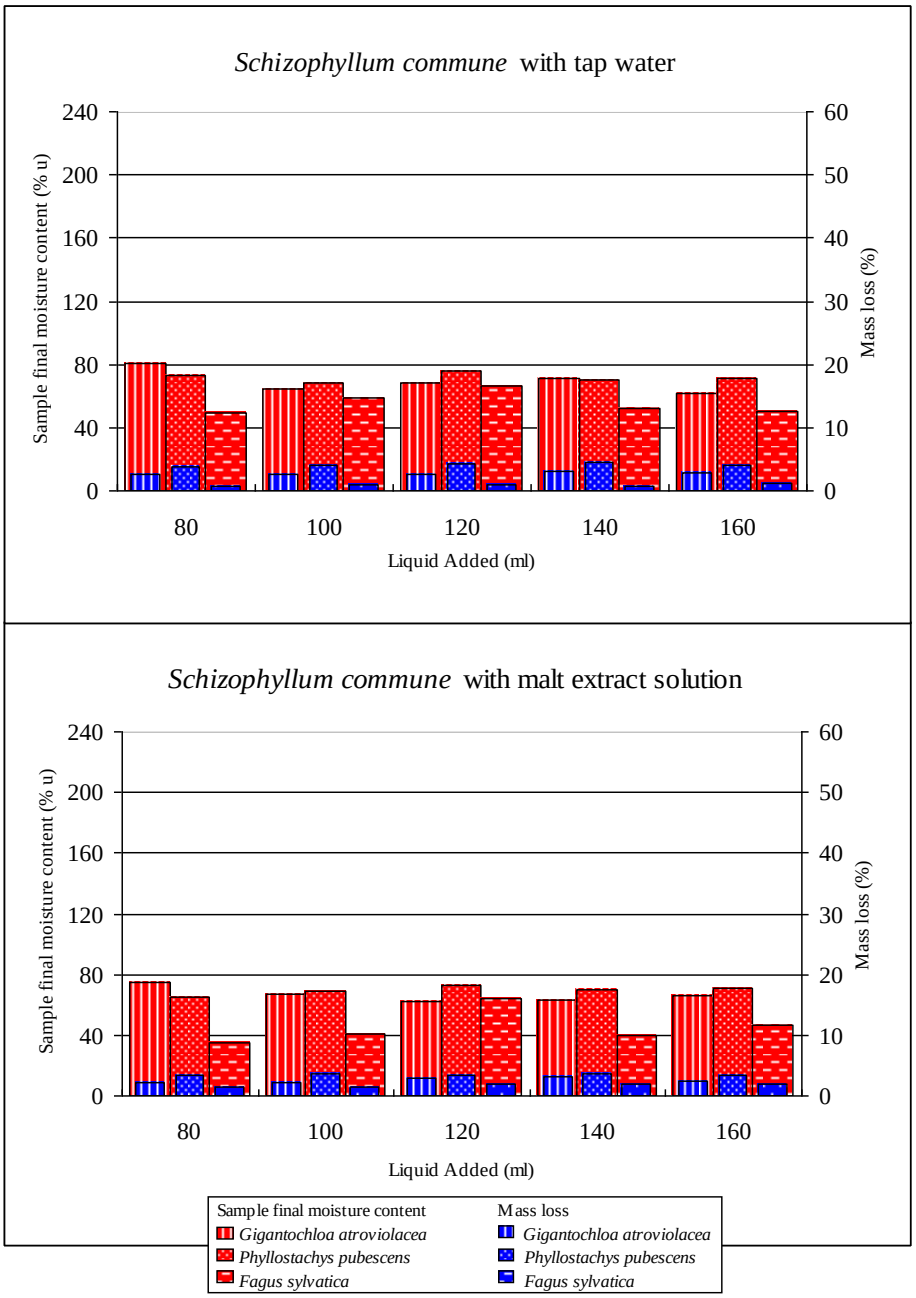

Fig. 3.17. Influence of moisture content and nutrients on mass loss by *Schizophyllum commune*.

Maximum ML of bamboo was only 4.6 %. Thus, possible influences among the parameters, bamboo species, moisture content and nutrient addition, were rather indistinct for this fungus.

This low activity corresponds to the results on agar in preserving jars (Chapter 3.4.1) and in Kolle flasks (chapter 3.4.3). Also the beech wood samples revealed negligible decay (0.7 %) by *S. commune*, which corresponds to previous results with wood samples (Schmidt and Liese 1978).

Fig. 3.18 shows the results for *C. puteana.*

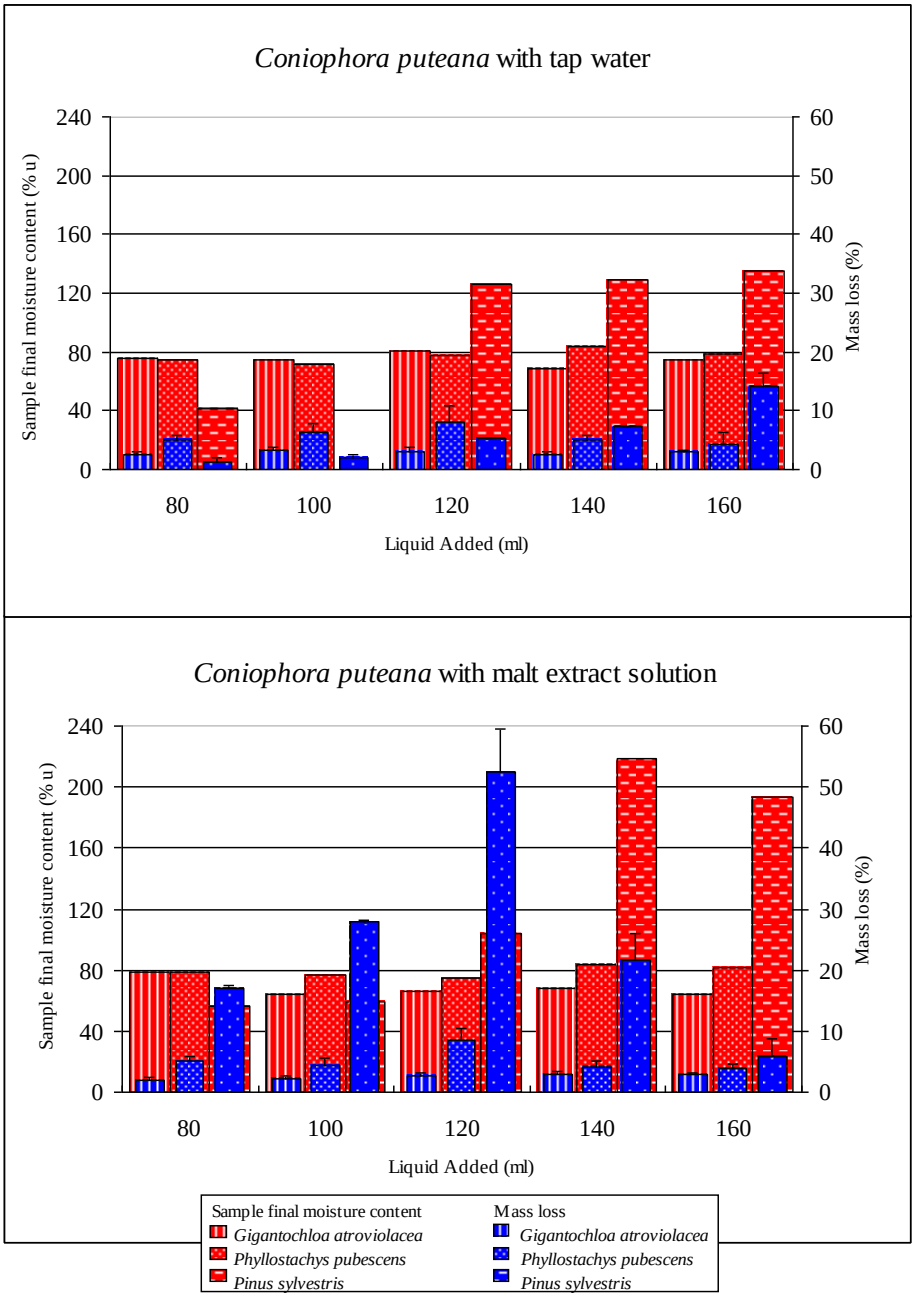

Fig. 3.18. Influence of moisture content and nutrients on mass loss by *Coniophora puteana*.

Maximum ML of bamboo was 8.4 %, whereby *P. pubescens* was more decayed than *G. gigantochloa*. Both additions, tap water and malt extract, increased ML from the 80 to 120 ml

liquid addition, followed by decrease to 160 ml liquid content. Scots pine wood revealed significant differences among the liquid additions: With tap water, decay increased stepwise to 14.0 % maximum at 160 ml water addition. The highest amount of decay displayed a maximum 52.5 % ML at 120 ml malt extract addition, which produced 104 % u moisture content of vermiculite. The subsequent decrease of ML may be caused by too much water in the vermiculite.

The standard deviation is not given in Fig. 3.17 due to the only small mass loss by *S. commune*), but for *C. puteana* (Fig. 3.18) due to its greater decay capacity. Both bamboo species *G. atroviolacea* and *P. pubescens* were rather resistant to degradation by S. *commune* and *C. puteana* over the 32 weeks of incubation, the first bamboo being more resistant than the latter one. Adding different amounts (80 to 160 ml) of water or nutrient solution to vermiculite had only a small effect. However, the final bamboo moisture contents were 61 to 84%, which is a suitable range for decay fungi (Schmidt 2006).

Fungi are able to transport water by their mycelia from a moisture source to neighbouring wood (Schmidt 2006). Figs. 3.17 and 3.18 show that the final moisture content of the bamboo samples was only slightly influenced by the initial liquid additions. Obviously, both fungi did not transport water from vermiculite to the samples. The presence of the metal ring between vermiculite and sample could not be the reason because *C. puteana* could moisten the *P. sylvestris* wood. Fig. 3.18 (bottom) shows that moisture content in Scots pine increased up to 218 % u parallel to water addition. Subsequently, ML decreased because of too much water in the wood.

To investigate if the fungi were still alive after 32 weeks of incubation, the vitality test performed proved living mycelium in all culture vessels (Fig. 3.19). Because fruit bodies did not grow in the jars and because both fungi do not produce asexual spores, survival was not due to spores. Thus, it can be deduced that mycelia were active over the whole culture period.

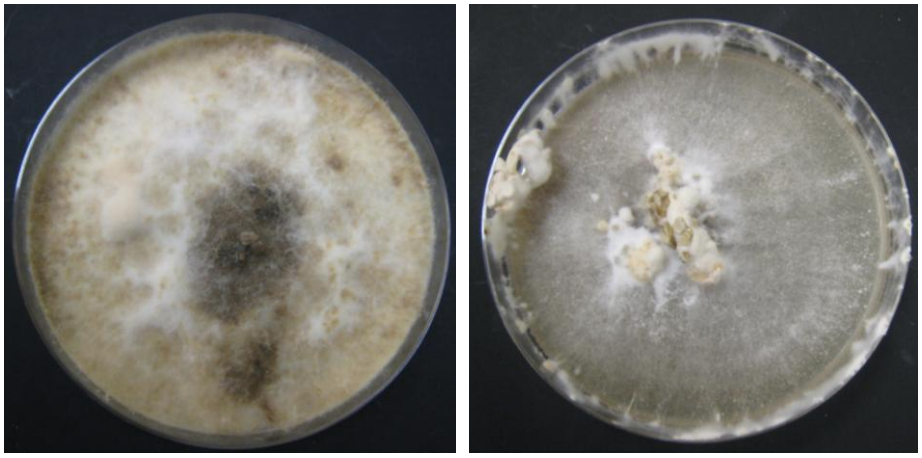

Fig. 3.19. Vitality test on malt extract agar after vermiculite incubation.
Left: *Coniophora puteana*; right: *Schizophyllum commune*.

Schmidt et al. (2011) assumed that high bamboo ML by *C. puteana* and *S. commune* in the Fungus cellar test was mainly caused by the moisture conditions and much less by components from the unsterile soil. The vermiculite test as a pure-culture experiment in closed vessels did not show a significant effect of moisture content and nutrient addition on bamboo degradation. Thus, bamboo degradation in nature may be also influenced by better air conditions, soil minerals or vitamins from soil bacteria. Already Leutritz (1946) demonstrated that soil serves as a water holding substrate and provides substances from the soil. The influence of minerals and vitamins on soft rot was reported by Worrall and Wang (1991) and Worrall et al. (1991). However, the small differences of mass loss among the bamboos, fungi and test parameters make a deeper discussion senseless.

The fungi had been selected due to three reasons: *S. commune* is very common on bamboo and the experimental strain was our own isolate from bamboo; *C. puteana* is an obligatory test fungus in the European standard EN 113; third, it was hoped to explain the influence of moisture content and nutrients in the fungus cellar test.

However, with regard to wood, the decay results with *C. puteana* and *P. sylvestris* samples (Fig. 3.18) show the suitability of the proposed technique for degradation studies. Adding 2 % malt extract solution until a wood moisture content reached approximately 100 % provided high ML within 32 weeks of incubation. The vermiculite method will most likely produce significant bamboo decay as well if more aggressive fungi such as *P. ostreatus* and *T. versicolor* are used.

3.4.5 Field test

A comparative durability test of *Quercus petraea* and *Q. robur* samples revealed for EN laboratory tests the durability class II (durable), but only class IV (somewhat durable) in the above-ground test and class V (non-durable) in the in-ground examination, respectively (Brischke et al. 2009). To compare the bamboo laboratory results (see Table 3.10) to the field, a small stake test was performed for 3.5 years. Examples of the stakes from *Bambusa vulgaris* and three unknown species are shown in Fig. 3.20.

Fig. 3.20. Examples of stakes from the field test.

Fungal activity on the stakes was visually assessed according to the rating of Papadopoulos (2010). Table 3.12 summarizes the results.

Table 3.12. Visual evaluation of susceptibility of bamboo stakes to fungi after 3.5 years field test

Stake part		*Bambusa vulgaris*	Species 3	Species 5	Species 10
Above ground	top	2	4	3	3
	middle	2	4	3	2
	bottom	2	3	2	2
In ground	top	2	3	2	2
	middle	1	2	1	1
	bottom	1	1	0	1

The above-ground parts of the stakes revealed no attack (rating 4) to moderate attack (rating 2), the in-ground areas showed slight attack (3) to total destruction (0). Fungal activity varied between the species and sample regions. Among the bamboos, the unknown species 3 was most resistant and no. 5 was most susceptible to fungi.

For more information, the below-ground parts were opened to view the inner of the stakes (Fig. 3.21).

Fig. 3.21 Cross-sectional view of opened stakes from the below-ground part. Left: *Bambusa vulgaris*; right: species 10.

The opened stakes showed different stagess of degradation. Some revealed a thin outer layer of soft-rot decay which is characteristic for lignocelluloses in soil or wet conditions (Liese 1985, Schmidt 2006). In the centre, the presence of white rot was assumed due to the brightened discolouration of the tissue. Although there is rather limited data of the performed field tests, the results underline the well-known fungal susceptibility of bamboos in ground contact (e.g., Liese 1985).

3.5 Chemical analysis of degraded bamboo

To obtain knowledge on the specific attack of the various rot fungi, the chemical composition of the bamboo cell walls was performed. Samples used for the analyses derived from the preserving jar test (Chapter 2.4.1). Three bamboo species, after 1 year degradation including control samples, were analyzed. All rot types, different decay fungi and increasing ML of samples were considered (Table 3.13).

Table 3.13. Characterization of bamboo samples for chemical analyses

Bamboo	Fungi	Rot type	Mass loss (%)
Phyllostachys pubescens	Control		
	Coniophora puteana	brown	4.7
	Schizophyllum commune	white	5.2
	Gloeophyllum trabeum	brown	5.3
	Pleurotus ostreatus	white	21.0
	Chaetomium globosum	soft	38.0
	Trametes versicolor	white	47.8
Gigantochloa atroviolacea	Control		
	Coniophora puteana	brown	5.6
	Gloeophyllum trabeum	brown	5.7
	Schizophyllum commune	white	6.7
	Trametes versicolor	white	51.6
Bambusa maculata	Control		
	Pleurotus ostreatus	white	28.2
	Chaetomium globosum	soft	31.8
	Trametes versicolor	white	62.5

For sugar analyses, the borate complex anion exchange chromatography (HPAEC-borate) analysis using cubicinchoninate for detection was applied for the direct quantification of underivatized sugars. HPAEC-borate requires a strong anion exchange gel as stationary phase. Applying a linear gradient between 0.3 M and 0.9 M potassium borate buffer pH 9.2 allows the separation of the wood sugars as their borate complexes. Due to the high borate concentration, the column continuously regenerates itself so that acid hydrolysates can be directly injected without neutralization. In a large range, the colour development, measured at

560 nm, is proportional to the sugar concentration (Sinner and Puls, 1978). The wood sugars including 4-O-Me-glucuronic acid elute within 50 min.

As an example, the results of the chromatogram of the *P. pubescens* control hydrolysate are presented in Fig. 3.22.

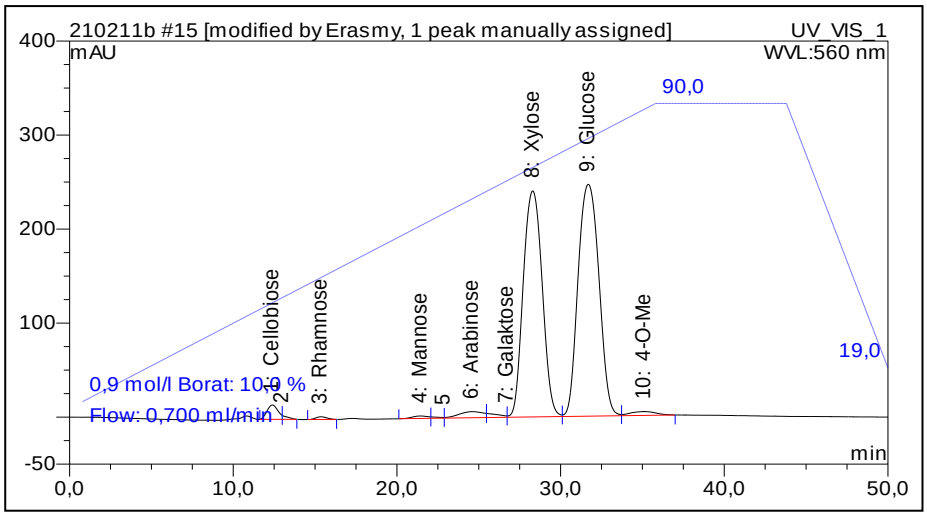

Fig. 3.22. HPAEC-borate chromatogram of *Phyllostachys pubescens* control hydrolysate.

All bamboo samples were measured three times and the average was calculated. The amount of sugars in the *P. pubescens* control is listed in Table 3.14.

Table 3.14. HPAEC-borate analysis of the *Phyllostachys pubescens* control

Peak no.	Component	Retention time (min)	Area mAU*min	Height mAU	Amount (%)
1	Cellobiose	12.39	11.71150	15.22644	0.74
2	n.a.*	13.00	1.57442	4.11063	n.a.
3	Rhamnose	15.33	2.24589	2.85388	0.11
4	Mannose	21.45	2.91764	2.64550	0.20
5	n.a.	22.85	0.88235	0.73170	n.a.
6	Arabinose	24.59	11.06571	6.51314	1.31
7	Galactose	26.72	3.72431	1.42916	0.25
8	Xylose	28.30	323.37909	240.38193	23.85
9	Glucose	31.69	364.64658	246.60522	40.51
10	4-O-Me**	35.11	7.49265	4.06662	0.51

* not analyzed; ** 4-*O*-methylglucuronic acid.

Chemical analysis of *Bambusa balcooa* (Vena et al. 2013) revealed for the healthy tissue 7.1 % extractives, 54.6 % glucan, 21.6 % xylan, 1.1 % arabinan, 25.2 % Klason lignin and 2.4 % ash.

Table 3.15 summarizes the results of the chemical analyses of samples after 1 year degradation. Lignin content was determined by hydrolysis. After two step hydrolysis, the lignin amount was determined gravimetrically. The first three analyses columns contain the results for dry matter; acetone and ethanol extract, which are included by routine in those overviews, but are of lower significance for the matter in hand.

The percent loss of chemical constituents after 1 year of degradation is given in Table 3.16.

Table 3.15. Chemical composition (%) of bamboos after 1 year of fungal degradation

Bamboo	Fungi	Dry weight	Acetone extract	Ethanol extract	Hemicellulose							Cellulose			Lignin
					4-*O*-methyl-glucuronic acid	Arabi-nose	Galac-tose	Man-nose	Rham-nose	Xylose	Total	Cello-biose	Glucose	Total	
Phyllostachys pubescens	Control	95.05	4.61	1.05	0.52	1.37	0.26	0.19	0.11	24.38	26.82	0.69	41.50	42.19	26.25
	Coniophora puteana	94.87	3.45	0.80	0.40	1.19	0.24	0.20	0.10	23.86	25.99	0.21	41.11	41.32	25.64
	Schizophyllum commune	94.52	2.70	0.65	0.39	1.05	0.21	0.19	0.09	23.09	25.03	0.21	39.68	39.89	25.81
	Gloeophyllum trabeum	94.38	2.83	0.60	0.39	1.14	0.33	0.20	0.09	23.65	25.80	0.21	41.02	41.23	25.88
	Pleurotus ostreatus	94.44	2.42	0.79	0.32	0.80	0.16	0.19	0.07	18.68	20.22	0.19	35.34	35.53	20.91
	Chaetomium globosum	94.35	4.24	1.39	0.24	0.65	0.19	0.17	0.06	14.05	15.36	0.11	22.62	22.73	20.63
	Trametes versicolor	94.45	3.34	0.96	0.19	0.55	0.10	0.15	0.05	12.20	13.24	0.11	24.24	24.35	14.09
Gigantochloa atroviolacea	Control	94.42	3.52	1.34	0.46	1.13	0.61	0.10	0.09	16.14	18.53	0.73	47.86	48.59	29.46
	Coniophora puteana	94.44	1.98	0.94	0.36	0.88	0.50	0.11	0.08	15.69	17.62	0.21	47.74	47.95	27.69
	Gloeophyllum trabeum	94.55	2.06	0.74	0.38	1.05	0.59	0.12	0.08	16.04	18.27	0.21	45.95	46.16	27.91
	Schizophyllum commune	94.24	2.05	0.74	0.38	0.99	0.49	0.11	0.08	15.62	17.67	0.21	46.54	46.75	28.34
	Trametes versicolor	94.68	1.70	1.03	0.16	0.43	0.18	0.11	0.03	7.09	8.00	0.12	25.01	25.12	14.37
Bambusa maculata	Control	94.65	3.05	1.20	0.41	0.95	0.50	0.10	0.08	18.13	20.17	0.54	52.15	52.69	26.43
	Pleurotus ostreatus	94.95	2.06	1.36	0.21	0.45	0.19	0.07	0.05	11.36	12.33	0.20	40.53	40.73	16.54
	Chaetomium globosum	93.74	3.53	1.23	0.22	0.66	0.28	0.15	0.06	12.12	13.49	0.14	28.90	29.04	23.32
	Trametes versicolor	94.70	2.46	1.33	0.12	0.24	0.11	0.10	0.03	5.69	6.29	0.09	20.75	20.84	9.41

Table 3.16. Percent loss of chemical constituents of bamboos after 1 year of fungal degradation

Bamboo	Fungi	Rot type	Component loss (%)			
			Mass	Hemicellulose	Cellulose	Lignin
Phyllostachys pubescens	Control					
	Coniophora puteana	brown	4.7	3.09	2.06	2.32
	Schizophyllum commune	white	5.2	6.67	5.45	1.68
	Gloeophyllum trabeum	brown	5.3	3.80	2.28	1.41
	Pleurotus ostreatus	white	21.0	24.61	15.79	20.34
	Chaetomium globosum	soft	38.0	42.73	46.12	21.41
	Trametes versicolor	white	47.8	50.63	42.28	46.32
Gigantochloa atroviolacea	Control					
	Coniophora puteana	brown	5.6	4.91	1.32	6.01
	Gloeophyllum trabeum	brown	5.7	1.40	5.00	5.26
	Schizophyllum commune	white	6.7	4.64	3.79	3.80
	Trametes versicolor	white	51.6	56.83	48.30	51.22
Bambusa maculata	Control					
	Pleurotus ostreatus	white	28.2	38.87	22.70	37.42
	Chaetomium globosum	soft	31.8	33.12	44.89	11.77
	Trametes versicolor	white	62.5	68.82	60.45	64.40

The sugar composition of hemicellulose (Table 3.15) mainly comprising xylose, arabinose and 4-*O*-methylglucuronic acid agrees to the results by Vena et al. (2013) that bamboo hemicellulose is of the xylan-type, similar as in hardwoods.

Pleurotus ostreatus and *T. versicolor* (Table 3.16) consumed the three cell wall components hemicellulose, cellulose and lignin to nearly the same extent which is characteristic of white-rot fungi of the simultaneous type (Schmidt 2006). Maximum lignin degradation of 64% occurred by *T. versicolor* in *B. maculata*. Due to the low mass loss of 5.2 to 6.7% by the less aggressive fungus *S. commune* (Table 3.11) there were only minor changes to the hemicelluloses, cellulose and lignin content, respectively.

The less destructive brown-rot fungi *C. puteana* and *G. trabeum* (Table 3.11) revealed slight changes in the cell wall components. *G. atroviolacea* displayed remarkably high lignin degradation values relative to the other brown-rot fungi.

The soft-rot fungus *Ch. globosum* preferentially consumed the carbohydrates, which is characteristic of this rot type. However, there was also some decrease in lignin content, which may be due to the cleavage of methoxy groups from the aromatic rings by these fungi (Schmidt 2006).

3.6 Transmission electron microscopy (TEM) studies of degraded bamboo

For detailed knowledge on the micromorphology of bamboo cell wall degradation by fungi, samples from the degrading test in preserving jars (like as for chemical analyses, chapter 3.5) were investigated with the transmission electron microscope (TEM). Different degrees of degradation were used to group the results into early, medium and late decay stagess.

3.6.1 Early stages of decay

The TEM observations on early stages of decay (one month of incubation) of *Bambusa maculata* (Fig. 3.23) show that degradation was mostly confined to the parenchyma cells. Parenchyma cells with laminated wall layers (Liese 1998) contained hyphae in the lumen as well as in the cell wall. Fig. 3.23a shows hyphae eroding from one lumen through the wall in the neighbouring cell. The vessel (Fig. 3.23b) contained hyphae and holes in the secondary wall layer, this would occur as cavities in longitudinal sections, a unique characteristic of soft rot. The vessel seemed to be degraded more easily than the fibres. Most fibres in healthy bamboo were composed of 3-5 concentric layers. The secondary wall of some fibres near parenchyma cells consisted of more than 7 layers (Fig. 3.23c).

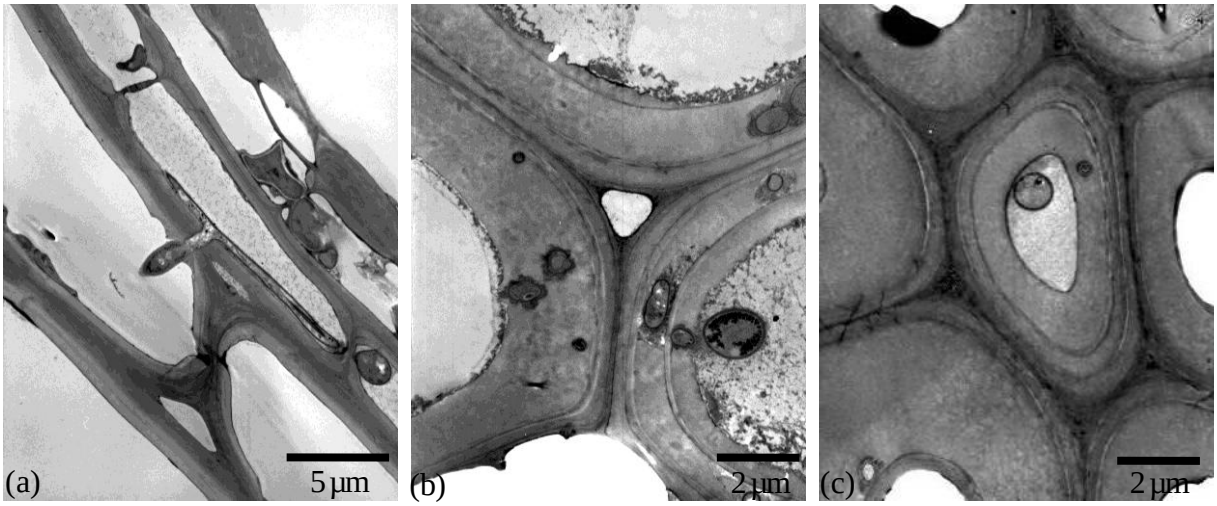

Fig. 3.23. Early soft-rot symptoms by *Chaetomium globosum.*
(a): parenchyma cells; (b): vessels with neighbouring parenchyma; (c): fibres.

3.6.2 Medium stages of decay

Fig. 3.24a, b shows medium stages of soft rot after 3 month of incubation. *Chaetomium globosum* degraded the cell corner between fibres, and the S_2 layer was decayed into many holes (cavities; Fig. 3.24a). The remaining compound middle lamella region (CML) and S_3 layer are shown in Fig. 3.24b.

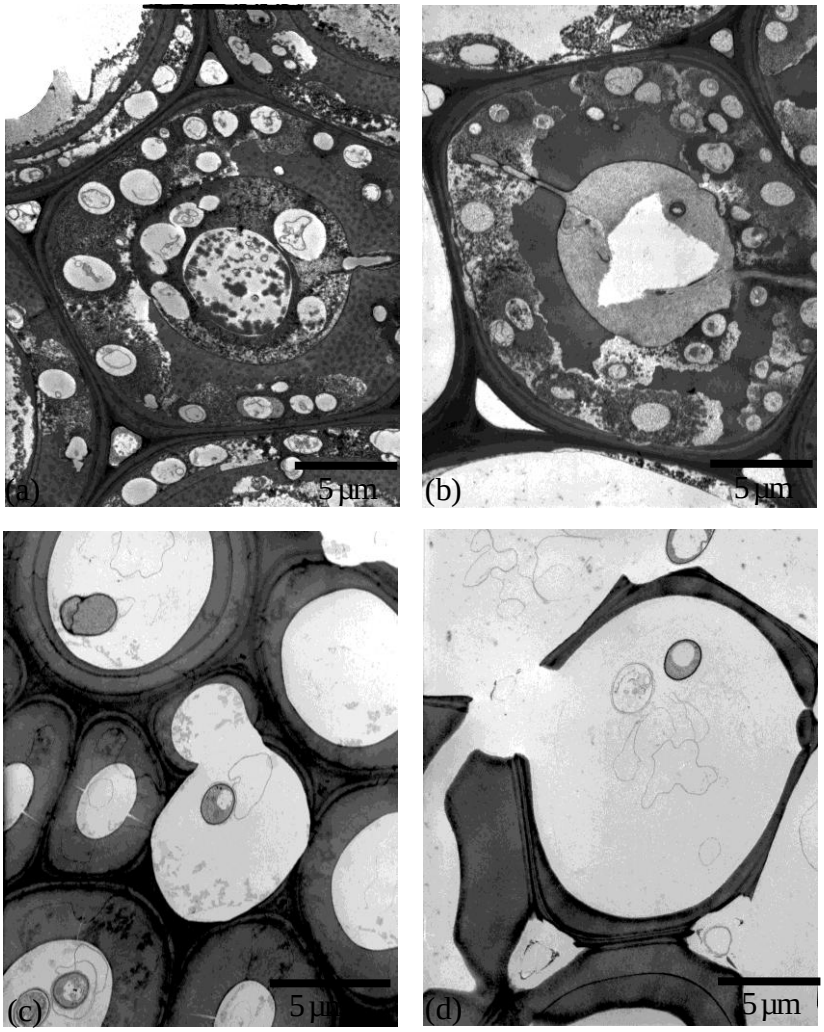

Fig. 3.24. Medium stages of decay by soft rot (*Chaetomium globosum*) and white rot (*Trametes versicolor*).

(a): many hyphae of *C. globosum* in the S_2 layer of a fibre and cell wall residues; (b): only some remaining S_2 layer residues in a fibre degraded by *C. globosum*; (c): secondary wall of fibres with erosion and wall decay after attack by *T. versicolor* with hyphae in the lumina; (d): parenchyma cells degraded by *T. versicolor*.

Generally, cell wall degradation by Basidiomycetes begins in the lumen (Liese 1970, Schmidt 2006). Fig. 3.24c shows the medium stage of white-rot attack by erosion and decay of the cell wall by *T. versicolor*. Hyphae migrated through pits, and the diameter of pits in parenchyma cells was enlarged (Fig. 3.24d).

3.6.3 Late stages of decay

In Fig. 3.25, late stages of soft- and white-rot decay after 12 months of incubation are presented. Fibres and parenchyma cells were severely decayed by soft-rot (Fig. 3.25 a, b). The S_2 layer was completely decayed. Only the CML and S_3 resisted attack, which is typically true for soft-rot attack. The white rot fungus *P. ostreatus* degraded the fibres completely so that only granular residues of the cells remained with some included hyphae.

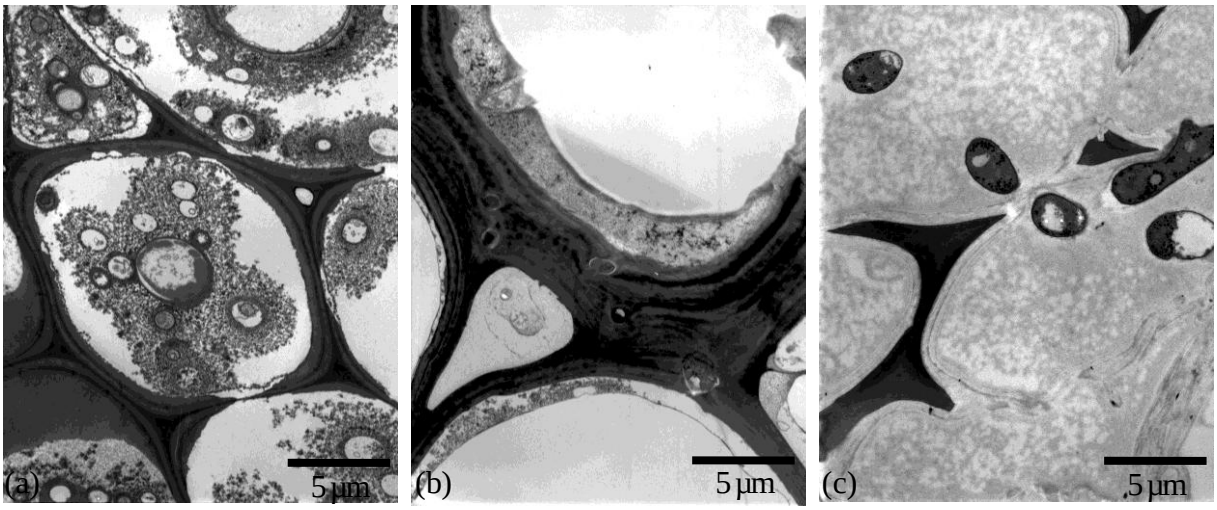

Fig. 3.25. Late stages of soft rot (*Chaetomium globosum*) and white rot (*Pleurotus ostreatus*). (a): fibres with totally degraded S_2 by *C. globosum*; (b): parenchyma cells with severe degradation by *C. globosum*; (c): fibres totally degraded by *P. ostreatu*s.

TEM studies on *P. pubescens* decayed by *L. edodes* (Kim et al. 2008) indicated that the CML in bamboo fibres was degraded at early stages of decay. This would indicate the selectivityof this type of white-rot (Liese 1998): *L. edodes* belongs to the simultaneous white-rot.

Brown-rot fungi were not included in the TEM investigations in this thesis study due to the low ML measured (chapter 3.5). To my knowledge, the only TEM investigation on brown-rot decay was performed by Cho et al. (2008) using *P. pubescens* and *G. trabeum*.

Altogether, the TEM studies on degraded bamboo revealed a rather similar micromorphology of cell wall decay as it occurs in wood (e.g., Liese 1998).

3.7 Ultraviolet microspectrophotometry (UMSP) studies of degraded bamboo

To investigate in situ the lignification of bamboo cell walls and fungal lignin degradation within them by white-rot fungi, UMSP studies were performed. Samples used derived from *Bambusa maculata* of the preserving jar test. The soft-rot fungus *Ch. globosum* was included for comparison. Table 3.17 lists characteristic ML of the investigated samples.

Table 3.17. Characteristics of *Bambusa maculata* samples used for ultraviolet microspectrophotometry

Fungus	Mass loss (%)		
	1 month	3 months	12 months
Cyathus stercoreus	0.2	1.6	17.4
Pleurotus ostreatus	0.8	5.8	28.2
Trametes versicolor	0.7	25.1	62.5
Chaetomium globosum	4.7	23.6	31.8

3.7.1 Light microscopy

Previously to the UMSP studies, sample sections were mapped out by light microscopy to determine the areas for subsequent UMSP (Fig. 3.26).

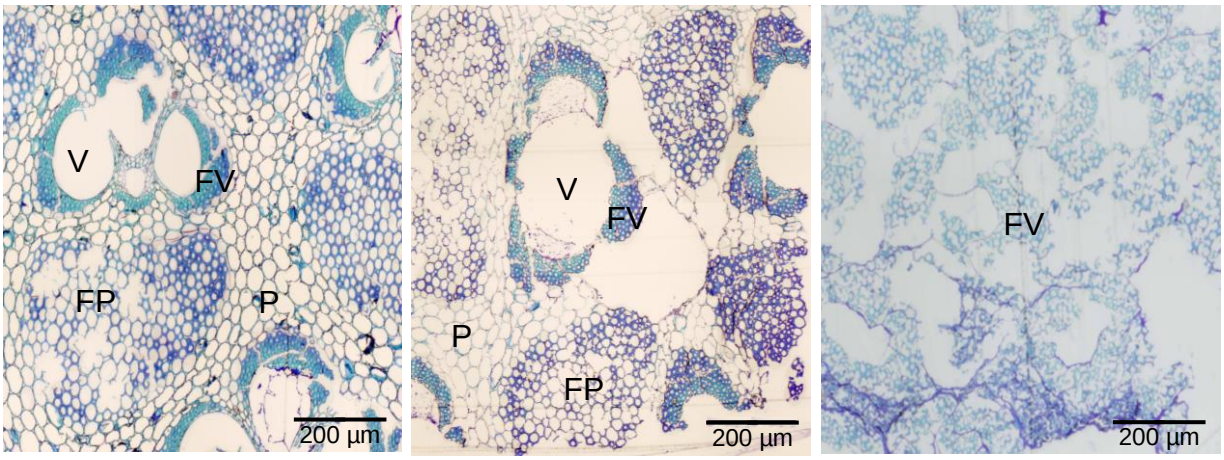

Fig. 3.26. Light microscopy of transverse section of healthy *Bambusa maculata* (left) and after degradation by *Trametes versicolor* for 3 months (middle) and for 12 months (right).
V: vessel, P: parenchyma, FV: fibres in the vascular bundle, F: fibres in ground parenchyma.

Additionally, Fig. 3.26 clearly demonstrates the progressive tissue degradation from healthy tissue via 3 months to 12 months incubations.

3.7.2 UV-absorbance spectra of individual cell wall layers

Generally, the lignin molecule exhibits an absorption maximum at 280 nm wavelengths and a broad shoulder between 310 and 320 nm (Fig. 3.27). This shoulder is typical for monocotyledons and can be linked to the presence of *p*-coumaroylation as demonstrated by Higuchi (1987).

The UV absorbance of a particular anatomical region depends both on the concentration of the various structural units of lignin, and the extinction coefficient of each structural unit. There is an absorbance maximum at 280–282 nm, which indicates the presence of the strong absorbing guaiacyl (G) lignin (Fergus and Goring 1970b, Musha and Goring 1975). The extinction coefficient of the G unit at 280 nm is 3.5 times that of the S (syringyl) unit (Fergus and Goring 1970a), and the extinction coefficient of the H (*p*-hydroxyphenylpropan) unit is lower than that of the G unit, but higher than that of the S unit (Faix and Schweers 1974).

The following figures show the absorbance curves between 240 and 400 nm wavelength.

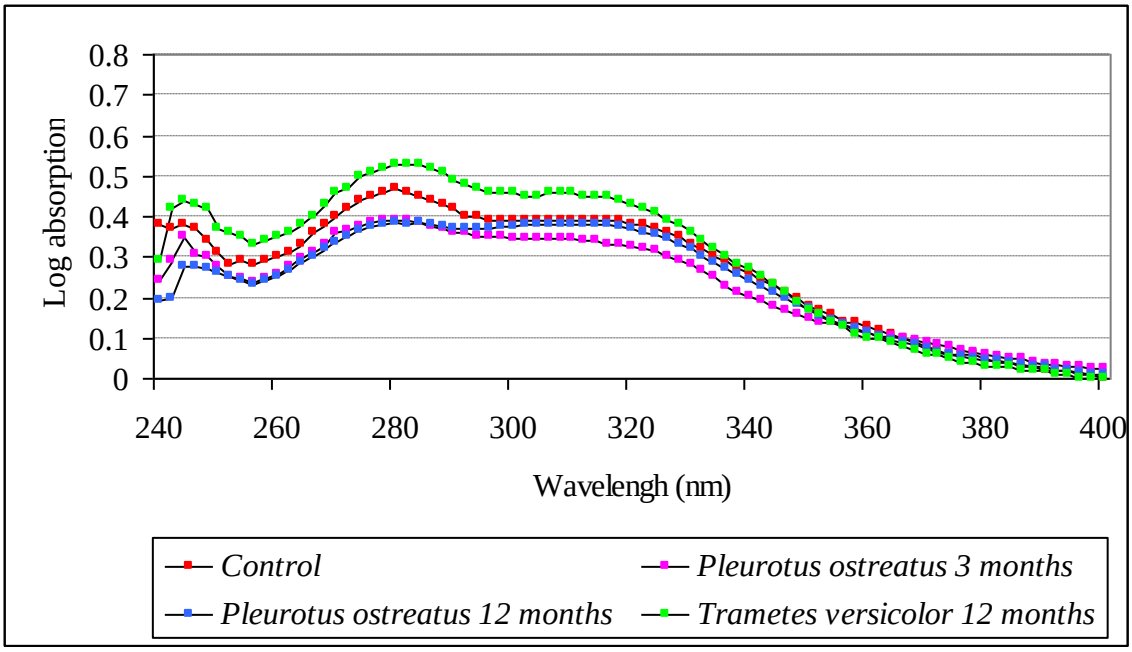

Fig. 3.27. UV-absorbance spectra of the secondary walls of fibres of *Bambusa maculata* samples.

Fig. 3.27 shows that the absorption at 280 nm decreases by the influence of the white-rot fungus *P. ostreatus*. The high absorption by *T. versicolor* may be due to the fact that the tissue after 12 month of incubation was strongly decayed (62.5 % mass loss) so that it had not been possible to select the correct cell wall layer. The UV spectra of the secondary wall of fibres have only a slight shoulder at 310-320 nm.

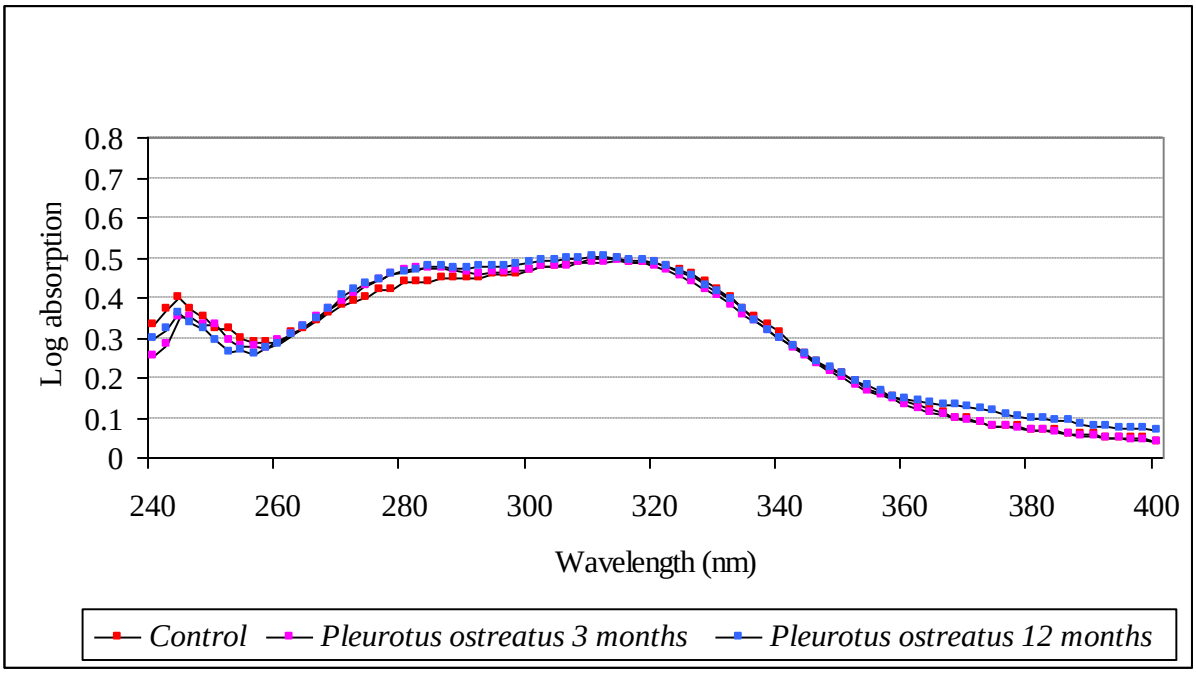

Fig. 3.28 UV-absorbance spectra of the compound middle lamella of thin-walled fibres within the ground parenchyma of *Bambusa maculata* samples.

The spectra of the compound middle lamella region (CML) of fibres within the ground parenchyma (Fig. 3.28) have the typical shoulders at 280-282 nm and 310-320 nm. These fibres have relatively thin secondary walls compared to the fibres in the vascular bundles. The spectra are rather similar. Obviously, there was no effect of the fungus on the lignin in the CML of these thin-walled fibres. The reason may be that *P. ostreatus* belongs to the simultaneous white-rot fungi whose enzyme activity occurs preferentially in the S_2 layer. No spectrum for *T. versicolor* was measured because there were no measurable remnants after culturing the fungus for 12 months.

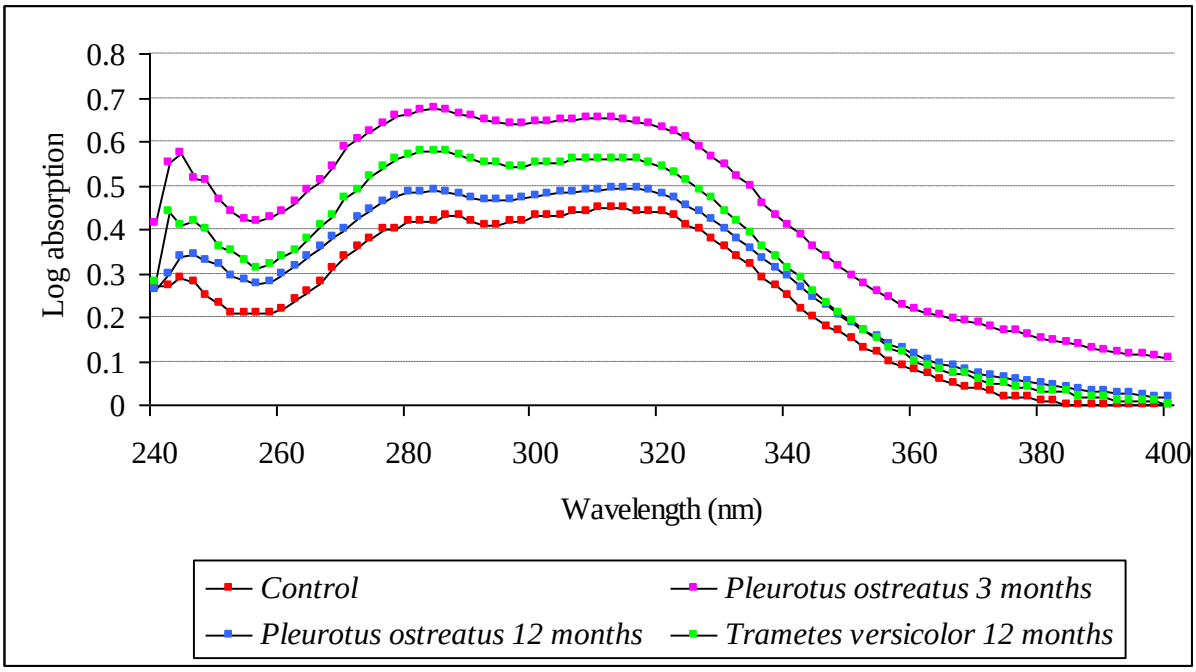

Fig. 3.29 UV-absorbance spectra of the compound middle lamella of thick-walled fibres in the vascular bundle of *Bambusa maculata* samples.

The spectra of the CML of thick-walled fibres in the vascular bundles (Fig. 3.29) with the two shoulders at 280-282 nm and 310-320 nm reveal a higher lignin content after fungal culture than in the control. An explanation may be that the content of carbohydrates in the CML of thick-walled fibres is higher than in thin-walled fibres. Fungi may have degraded these carbohydrates so that their relative lignin content increased.

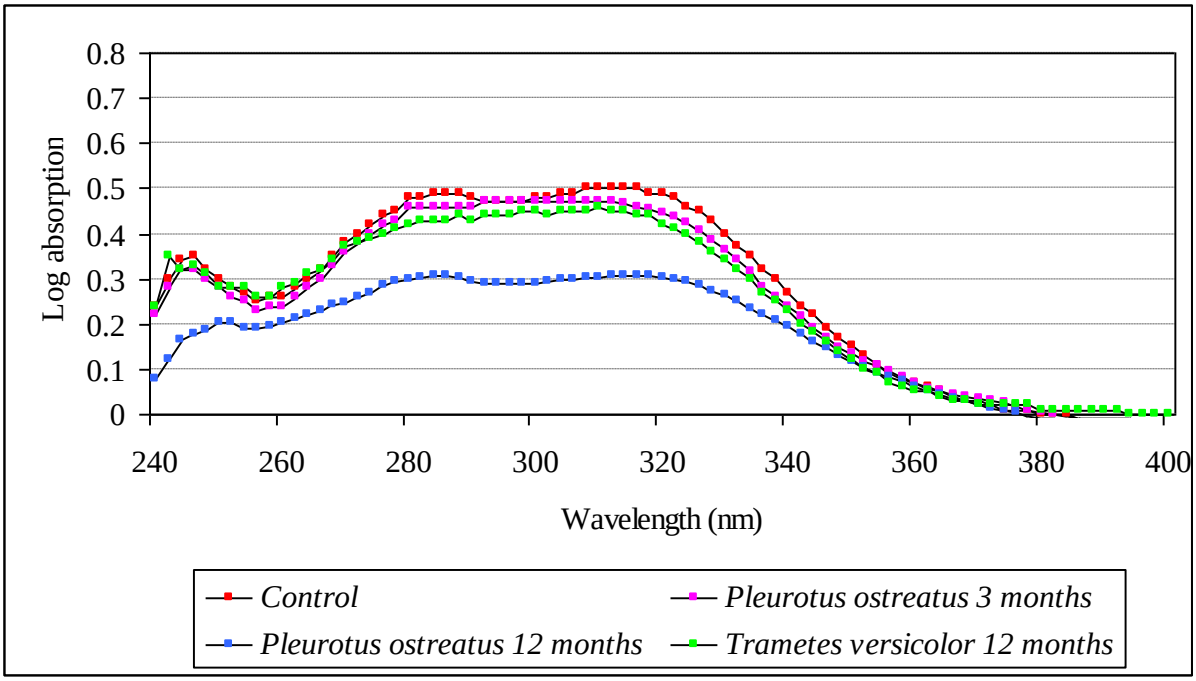

Fig. 3.30 UV-absorbance spectra of the secondary walls of vessels of *Bambusa maculata* samples.

The spectra of S_2 of vessel cell walls (Fig. 3.30) show a lignin decrease by both white-rot fungi. The absorbance decreased with incubation time of *P. ostreatus*.

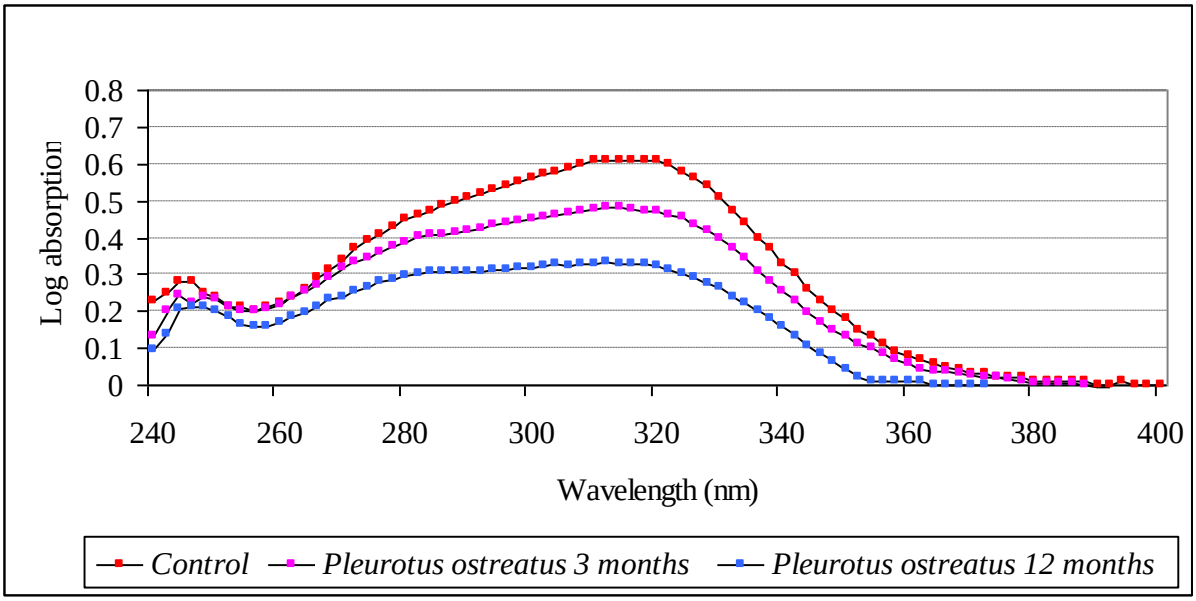

Fig. 3.31 UV-absorbance spectra of the compound middle lamella of parenchyma cells of *Bambusa maculata* samples.

The spectra of the CML of parenchyma cells (Fig. 3.31) have only the shoulder of 310-320 nm. The typical peak at 280 nm is not present. The lignin content of parenchyma cells decreased with increasing mass loss. There were no parenchyma cells remaining after 12 months of incubation with *T. versicolor*.

3.7.3 Scanning UV-microspectrophotometry

Using the scanning mode of the ultraviolet microspectrophotometer, tissues can be scanned at the absorbance of 280 nm in situ for the degree of lignifications in different cell walls and wall layers.

In the following figures, typical two- and three-dimensional UV microspectrophotometric scanning profiles of lignin distributions in the various bamboo tissues of *Bambusa maculata* are visualized. The colour pixels indicate different intensities of UV absorbance at 280 nm. The high resolution (0.25 µm x 0.25 µm per pixel) enables the differentiation of UV absorbance within different cell wall layers (Koch et al. 2003a). The two-dimensional UV image profiles reveal the average lignin concentration in a respective area. The three-dimensional presentations allow an improved evaluation of the topochemical distribution of lignin within the individual cell wall layers, including more than 47,000 measuring points.

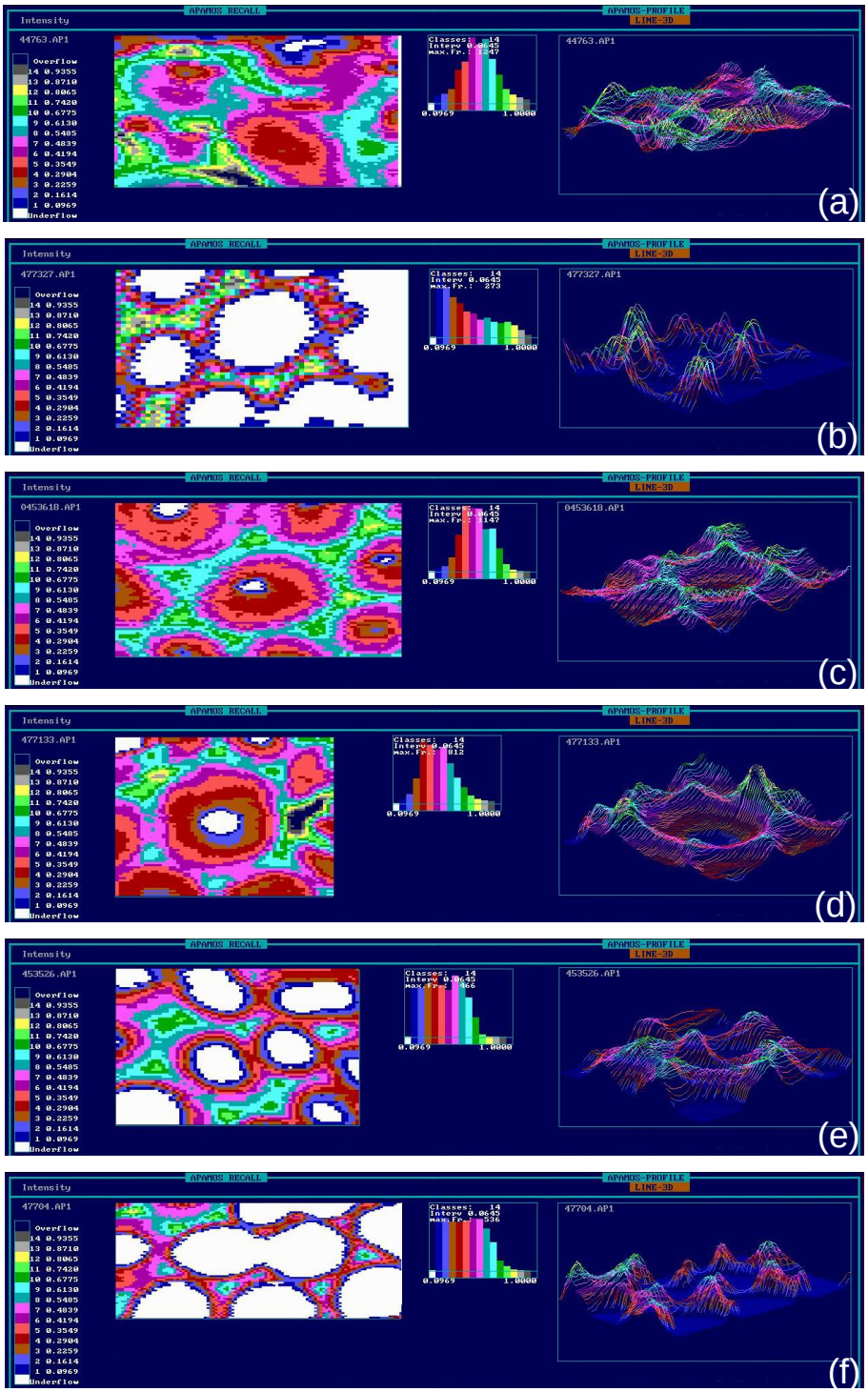

Fig. 3.32. UV micrographies and 3D profiles of thick-walled fibres in the vascular bundle of *Bambusa maculata* attacked by fungi measured at 280 nm.

(a): control; (b): *Cyathus stercoreus* 12 month; (c): *Pleurotus ostreatus* 3 months; (d): *P. ostreatus* 12 months; (e): *Trametes versicolor* 3 months; (f): *T. versicolor* 12 months.

Fig. 3.32 shows that the lignin content of the thick-walled fibres in the vascular bundle decreased with the decrease of ML produced by *C. stercoreus* and the two white-rot species. The delignification was higher in the S_3-S_2 wall layers. The cell corners were more lignified

than the S_2 layer. The image profiles of *P. ostreatus* 3 months and 12 months incubation differed only slightly, which does not correspond to different ML of 5.8 and 28.2 %, respectively (Table 3.17).

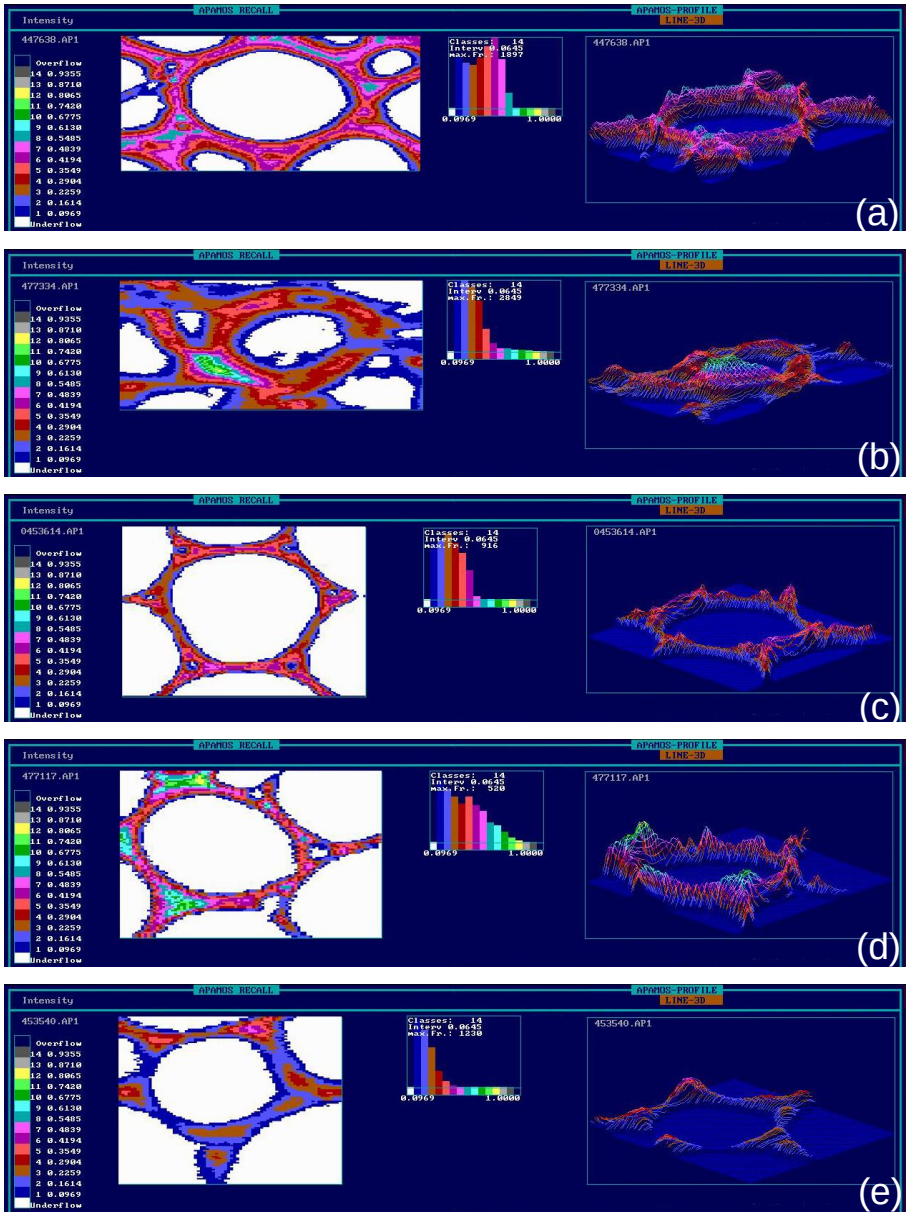

Fig. 3.33. 280 nm-UV micrographies and 3D profiles of thin-walled fibres within the ground parenchyma of *Bambusa maculata* attacked by fungi.
(a): control; (b): *Cyathus stercoreus* 12 month; (c): *Pleurotus ostreatus* 3 months; (d): *P. ostreatus* 12 months; (e): *Trametes versicolor* 3 months.

Like with the thick-walled fibres (Fig. 3.32), the lignin content of thin-walled fibres within the ground parenchyma decreased with degradation time/ML (Fig. 3.33). Obviously, delignification was parallel to ML. Fungal activity became very obvious already by the great

reduction of the wall thickness by the fungi, particularly visible at the *P. ostreatus* 3 months (34c) and 12 months incubations (34d), respectively. The twelve-months cultivation of *T. versicolor* was not measured because there were no measurable remnants.

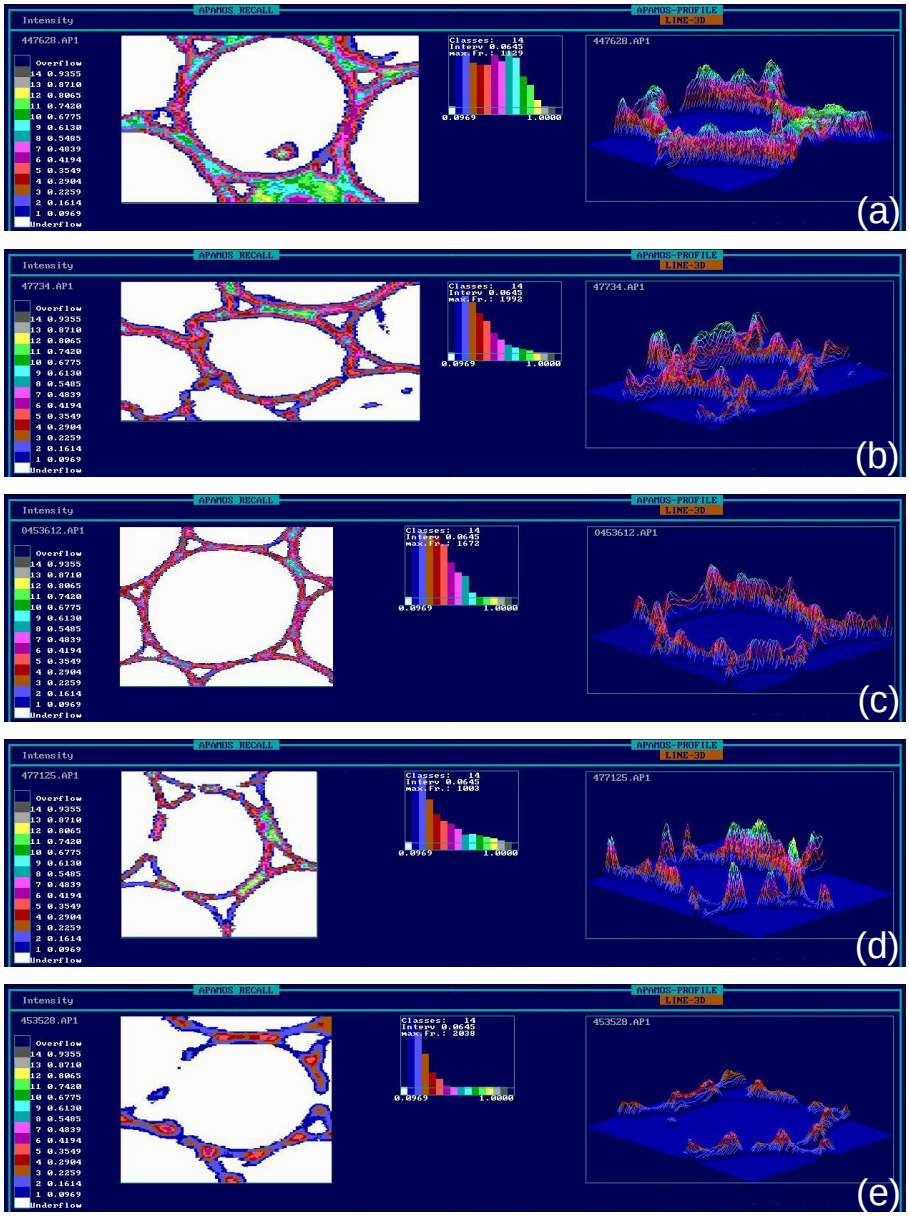

Fig. 3.34. 280 nm-UV micrographies and 3D profiles of parenchyma cell of *Bambusa maculata*.

(a): control; (b): *Cyathus stercoreus* 12 month; (c): *Pleurotus ostreatus* 3 months; (d): *P. ostreatus* 12 months; (e): *Trametes versicolor* 3 months.

The parenchyma cell walls revealed considerable decay and delignification by the fungi, particularly after incubation with both white-rot fungi (34c-e). Cell wall areas of adjacent cells were completely degraded (d, e) and the cell corners much less degraded.

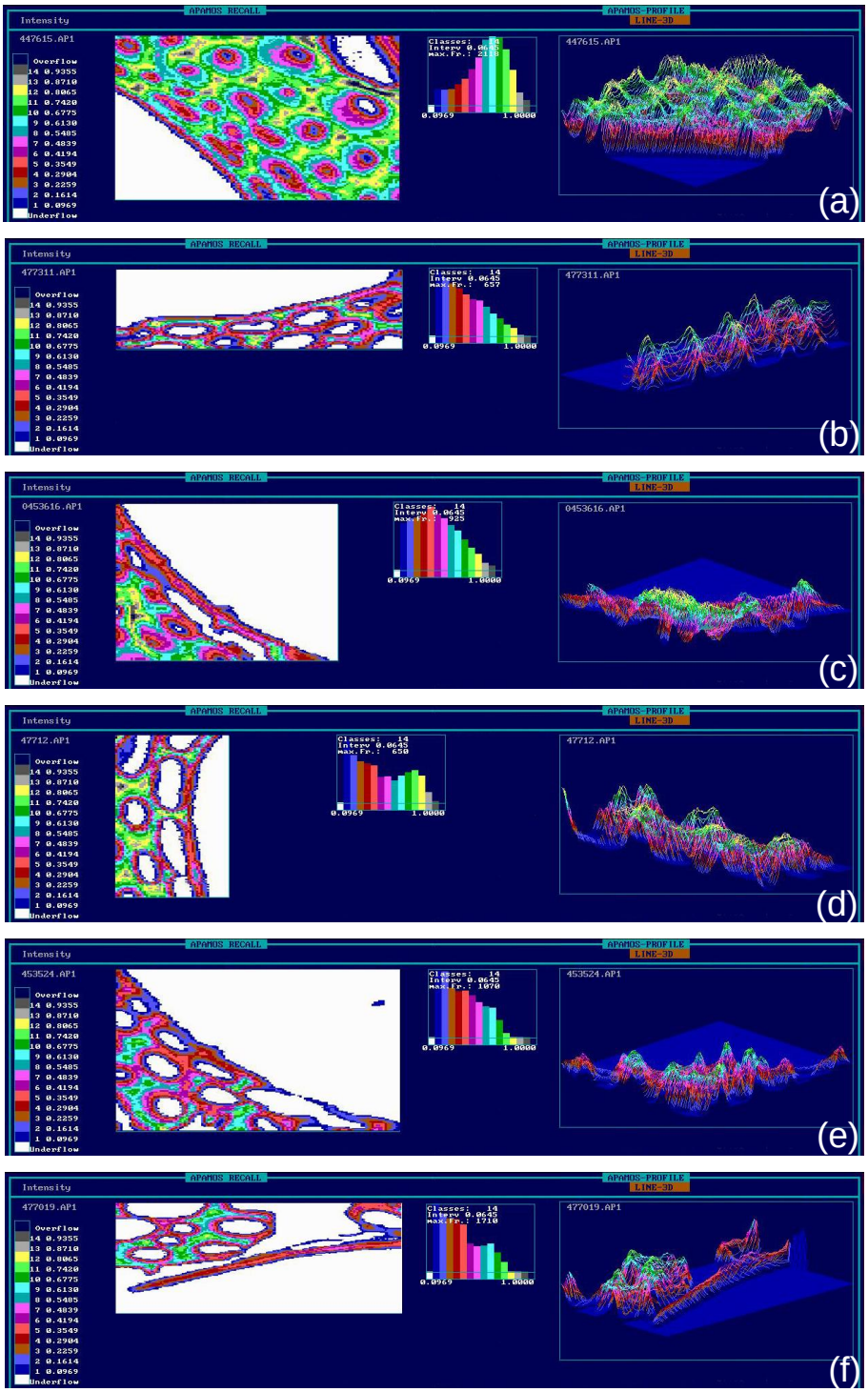

Fig. 3.35. 280 nm-UV micrographies and 3D profiles of the vessel-fibre area of *Bambusa maculata*.

(a): control; (b): *Cyathus stercoreus* 12 month; (c): *Pleurotus ostreatus* 3 months; (d): *P. ostreatus* 12 months; (e): *Trametes versicolor* 3 months; (f): *T. versicolor* 12 months.

The cell walls measured in the vessel-fibre area revealed a similar delignification as observed in Figs. 3.32 and 3.33 for the fibres. The vessel walls were also attacked (e, f).

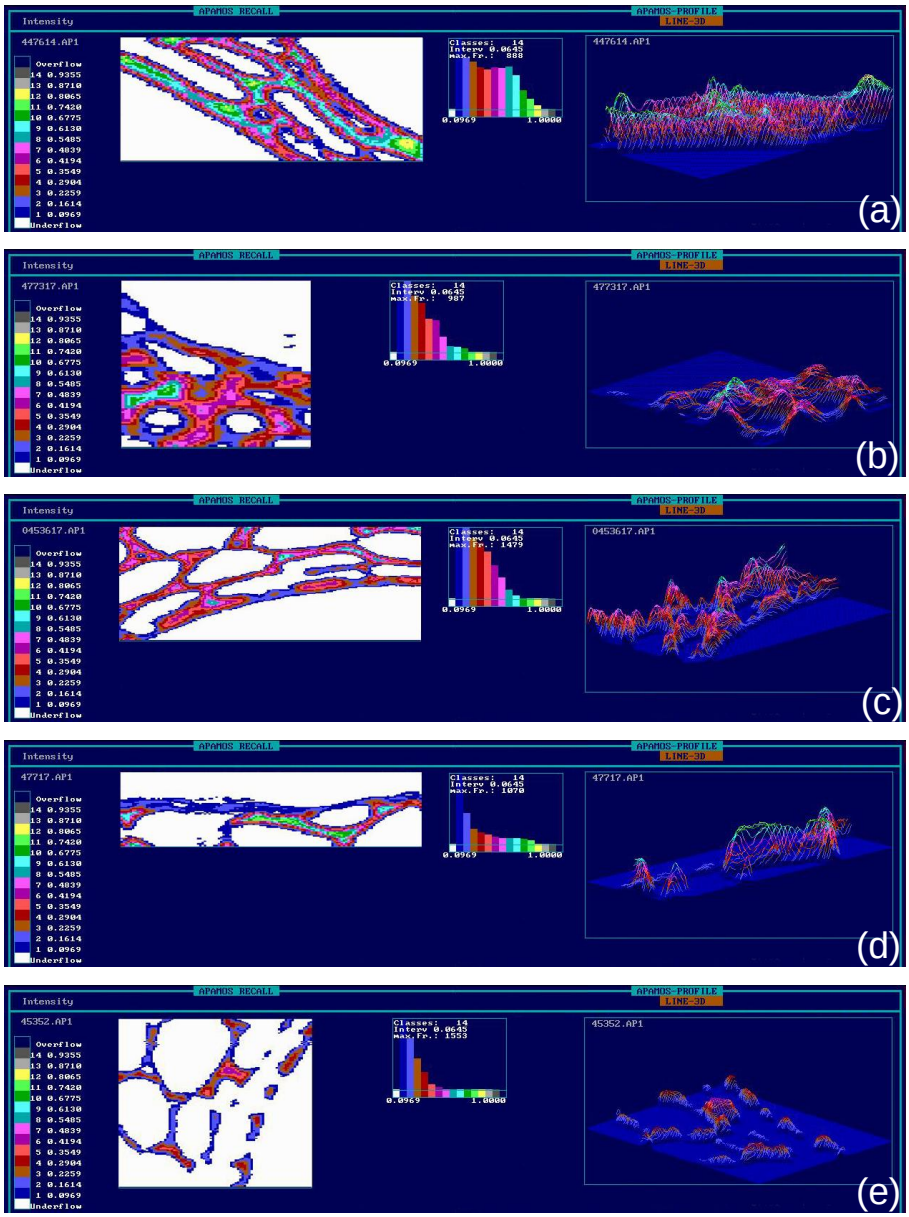

Fig. 3.36. 280 nm-UV micrographies and 3D profiles of the vessel-parenchyma area of *Bambusa maculata*.

(a): control; (b): *Cyathus stercoreus* 12 month; (c): *Pleurotus ostreatus* 3 months; (d): *P. ostreatus* 12 months; (e): *Trametes versicolor* 3 months.

The cell wall of the vessel and the surrounding parenchyma cells became delignified even in the early stages of degradation (3.36c).

Fig. 3.37 summarizes the results of the absorbance values at 280 nm of the different *Bambusa maculata* cell wall areas.

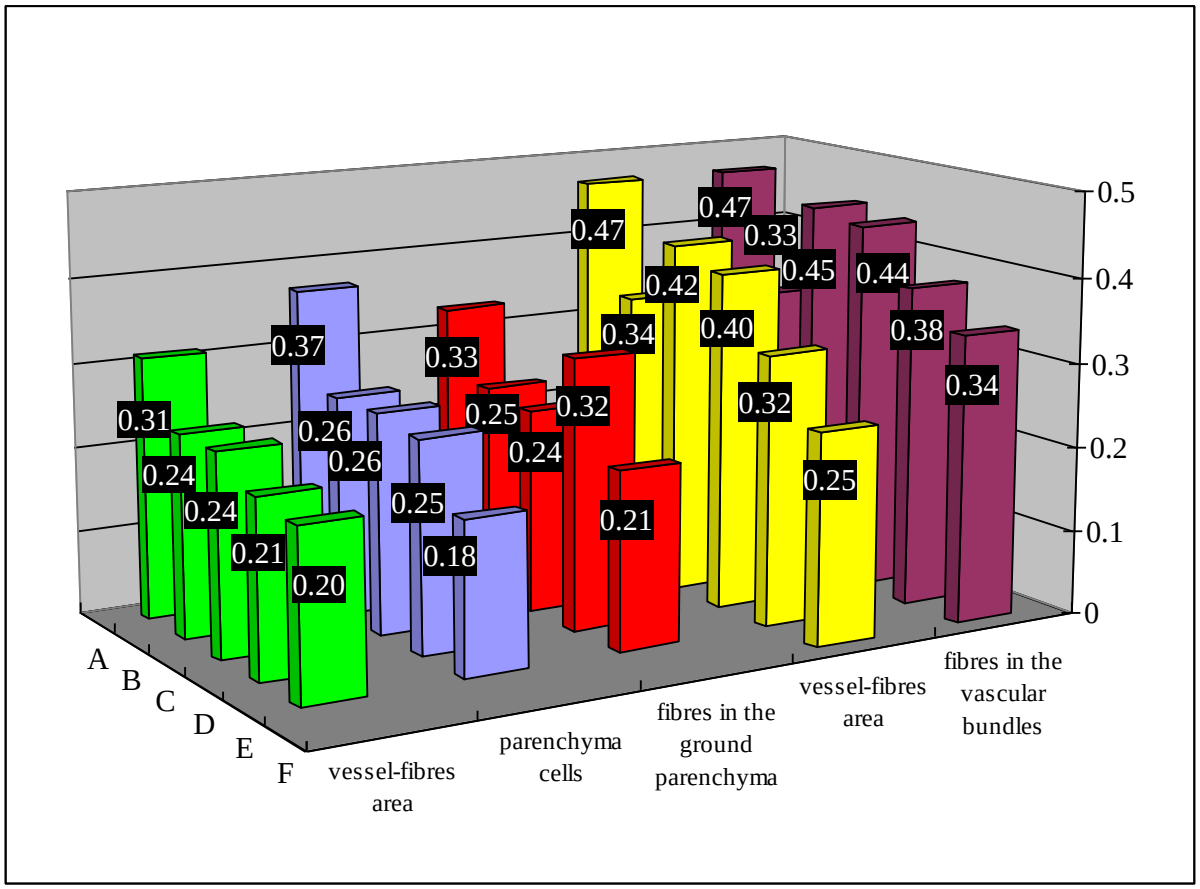

Fig. 3.37. Absorbance values at 280 nm wavelength of cell wall areas of *Bambusa maculata* attacked by fungi after different months of incubation.
A: control; B: *Cyathus stercoreus* 12 months; C: *Pleurotus ostreatus* 3 month; D: *P. ostreatus* 12 months; E: *Trametes versicolor* 3 months; F: *T. versicolor* 12 months.

Altogether, the UMSP results correspond to many former investigations, which revealed the suitability of the technique for studies on the distribution pattern of lignification/delignification within the cell walls of wood (Scott et al. 1969, Fergus et al. 1969b, Bauch et al. 1976, Koch and Kleist 2001, Takabe, 2002, Koch and Grünwald 2004, Singh et al. 2006) and bamboo (Lybeer and Koch 2005a, 2005b, Lybeer et al. 2006, Kim et al. 2008). The white-rot fungi investigated, *P. ostreatus* and *T. versicolor*, produce a simultaneous-type of white-rot. Their progressive degradation/delignification starting from the cell lumen towards the compound middle lamella region became visible in some UMSP

pictures. However, interpretation may become problematic when the measured wall area comprised different cell types like in the vessel/fibre and vessel/parenchyma region.

The topochemical analyses showed that lignin attack on lignin by simultaneous white-rot fungi occurs in parallel to ML. The beginning of delignification was evidenced at the outermost part of the secondary wall layer. UMSP proved that even at advanced decay the degradation was not homogeneous throughout the tissue, as there were still apparently less attacked cells among significantly damaged ones.

4 Conclusions

The bamboo culm is susceptible to infection and colonization by fungi during the first two months after harvest due to the fungus-suitable moisture content above the fibre saturation point. Consequently, culms after sea transport from Asia often show infection by moulds and other staining fungi. Moulds and Basidiomycetes were isolated from culms of several bamboo species from various countries after arrival in Germany. In total, 150 strains were isolated and 76 isolates were identified by rDNA-ITS sequencing. Most isolates were Deuteromycetes (moulds)/Ascomycetes. To aid in the identifications of closely related species from the genera *Aspergillus* and *Penicillium*, the ß-tubulin and calmodulin sequences were used for BLAST identification.

With regard to the discolouration of bamboo by blue-stain fungi, laboratory experiments showed that the samples were colonized by blue-stain fungi within four weeks of incubation. The bamboo tissue contained the typical thick, brown hyphae and chlamydospores of blue-stain fungi, as well the thin perforation hyphae, transpressoria, with which staining fungi penetrate lignified cell walls, similar to that observed in as it wood cells.

As is true for all lignocellulosic materials, bamboo (during use and storage) is susceptible to wood-rot fungi. Literature observations describe fungal attack on bamboo to be mainly white-rot Basidiomycetes and soft-rot Ascomycetes. These results identified the white-rot species *Schizophyllum commune* and the secondary saprobiont *Cyathus stercoreus* from imported culms. *S. commune* is often found on bamboos; however, the fungus revealed only low activity in decay tests.

To investigate the deterioration of bamboo culms by rot fungi, degradation studies were performed with samples of different bamboo species. Laboratory experiments included the EN 113 standard test in Kolle flasks and an agar test in preserving jars. The novel technique with vermiculite in preserving jars as a moisture and nutrient reservoir proved to be particularly suitable for wood samples as shown by the considerable mass loss of *Pinus sylvestris* wood by *Coniophora puteana*. The reason that the technique failed for bamboo may be due to the used fungi *C. puteana* and *S. commune*. Future studies should include other Basidiomycetes.

To imitate more natural conditions for fungi, larger-sized bamboo samples were tested in the "Fungus cellar", where the bamboo is inoculated with fungal pure cultures and placed on unsterile soil. In addition to the EN standard fungi, several white-, brown- and soft-rot fungi were used, along with different strains to consider strain variation.

The investigated bamboo species differed in their susceptibility to fungal deterioration. However, because the samples were derived from imported culms, important factors influencing the durability, like culm age, harvest season and position in the culm, were unknown. Future studies should consider these parameters.

With regard to the different groups of rot fungi, bamboo behaved rather resistant against brown rot, whereas soft-rot and white-rot fungi produced considerable deterioration.

As reason for the absence of brown-rot fungi among the isolations and for their low activity in the decay tests, it is assumed that the major bamboo hemicellulose is of the xylan-type, similarly as in hardwoods, which are the preferred wood substrates for white-rot fungi, whereas brown-rot fungi prefer softwoods with mannan as the hemicellulose. Soft-rot fungi have not been isolated because these fungi mainly occur in culms with soil and water contact. Correspondingly, a field test revealed soft-rot in the outer layer of the in-ground stakes. The stake centres showed white-rot.

For information on the enzymatic events during bamboo rotting, samples of known mass loss were subjected to chemical analyses. The investigated white-rot fungi *Pleurotus ostreatus* and *Trametes versicolor* consumed the three cell wall components (cellulose, hemicelluloses and lignin) almost uniformly at the same time and at a similar rate during the decay stages, which is characteristic of the simultaneous type of white-rot. Remarkable is the attack of the soft-rot fungus *Chaetomium globosum* on the lignin component, probably mainly due to demethylation of aromatic rings.

For microscopic information on the micromorphology of fungal attack, subcellular analyses of the cell tissue of the *Bambusa maculata* culm degraded by *C. stercoreus*, the white-rot fungi *P. ostreatus* and *T. versicolor* as well the soft-rot fungus *Ch. globosum* were carried out using light microscopy, TEM and UV microspectrophotometry. The TEM results provided

insight into morphological changes of the cell wall structure during decay. The UMSP area scans and additional point analysis measurements revealed insights into the topochemistry *in situ*. In particular, the latter technique gave information on the lignin modification process during cell-wall decomposition. The combination of TEM investigation and UV microspectrophotometry constituted a useful approach to determine topochemical variations of lignin distribution within individual cell wall layers.

Altogether, the results improved the basic view on the degradation of bamboo by fungi, which are needed for a better understanding of bamboo degradation by fungi.

5 References

Abdurachim, MRA 1964. Bamboo preservation in Indonesia. Rimba Indonesia, 9(1): 66-76.

Abdurachim, MRA 1975. Laboratory testing on the resistance of wood against fungi. Ministry of Agriculture, Jakarta, Indonesia.

Adan OCG, Samson RA. 2011. Fundamentals of molds growths in indoor environments and strategies for healthy living. Wageningen Academic Publishers, Wageningen, the Netherlands.

Adaskaveg JE, Blanchette RA, Gilbertson RL. 1991. Decay of date palm wood by white-rot and brown-rot fungi. Canadian Journal of Botany, 63(3): 615-629.

Alexopoulos CJ, Mims CW, Blackwell M. 1996. Introductory Mycology, 4th edn. Wiley, New York, United States.

Banerjee S, Mukhopadhyay S. 1962. A study on Merulius similis B. & FR. and the associated bamboo rot. Osterreichische Botanische Zeitschrift, 109(3): 197-212.

Bauch J, Seehann G, Fitzner H. 1976. Microspectrophotometrical investigations on lignin of decayed wood. Supplement 3 to Materials and Organisms, 141-152.

Becher G, Kaune P. 1966. Einflüsse beim Holzabbau durch Moderfäulepilze in Erde. Material und Organismen, 1(3): 201-220.

Blackwell M. 2011. The Fungi: 1, 2, 3 ... 5.1 million species? American Journal of Botany, 98 (3): 426–438.

Bravery AF, Berry RW, Carey JK, Cooper DE. 2003. Recognising wood rot and insect damage in buildings, 2nd edn. BRE, Watford, United Kingdom.

Breitenbach J, Kränzlin F. 1986. Pilze der Schweiz. Vol 2. Luzern, Switzerland.

Brischke C, Welzbacher CR, Gellerich A, Bollmus S, Humar M, Plaschkies K, Scheiding W, Alfredsen G, Van Acker J, De Windt I. 2013. Wood natural durability testing under laboratory conditions: results from around-robin test. European Journal of Wood and Wood Products, DOI 10.1007/s00107-013-0764-6.

Brischke C, Welzbacher CR, Rapp AO, Augusta U, Brandt K. 2009. Comparative studies on the in-ground and above-ground durability of European oak heartwood (*Quercus petrea* Liebl. and *Quercus robur* L.). European Journal of Wood and Wood Products, 67: 329-338.

Cho CH, Lee KH, Kim JS, Kim JS. 2008. Micromorphological characteristics of bamboo (*Phyllostachys pubescens*) fibers degraded by a brown rot fungus (*Gloeophyllum trabeum*). Journal of Wood Science, 54: 261-265.

Clark L. 2012. An updated tribal and subtribal classification of the bamboos (Poaceae: Bambusoideae). 9th World Bamboo Congress Proceedings, Antwerp, Belgium, pp 3-27.

Curling FC, Clausen CA, Winandy JE. 2002. Experimental method to quantify progressive stagess of decay of wood by basidiomycete fungi. International Biodeterioration & Biodegradation, 49(1): 13-19.

Dey S, Maiti T, Sreemany M, Ghosh TB, Bhattacharyya BC. 1992. Characterization of white-rotted and brown-rotted rice straw by X-ray photoelectron spectroscopy. Holzforschung, 46 (5): 385-390.

Djarwanto, Suprapti S. 2004. Laboratory testing on the resistance of wood against fungi. In Herjanto E et al. (Eds) Prosiding Pertemuan dan Presentasi Ilmiah Standardisasi. 11-12 October 2004, Jakarta, Indonesia, pp 15-22.

Donaldson LA. 1992. Lignin distribution during latewood formation in *Pinus radiate*. IAWA Bulletin ns, 12: 381-387.

Dugan FM, Braun U, Groenewald JZ, Crous PW. 2008. Morphological plasticity in *Cladosporium sphaerospermum*. Persoonia, 21: 9-16.

EN 113. 1996. Determination of the toxic values of wood preservatives against wood destroying Basidiomycetes cultured on agar medium. Beuth, Berlin, Germany.

EN 350-1. 1996. Durability of wood and wood-based products. Natural durability of solid wood. Part 1: Guide to the principles of testing and classification of natural durability of wood. Beuth, Berlin, Germany.

EN 350-2. 1997. Durability of wood and wood-based products. Natural durability of solid wood. Part 2: Guide to natural durability and treatability of selected wood species of importance in Europe. Beuth, Berlin, Germany.

EN 807. 2001. Wood preservatives - Determination of the effectiveness against soft rotting micro- and other soil inhabiting micro-organisms. Beuth, Berlin, Germany.

Faix O, Bremer J, Schmidt O, Stevanovic T. 1991. Monitoring of chemical changes in white-rot degraded beech wood by pyrolysis—gas chromatography and Fourier-transform infrared spectroscopy. Journal of Analytical and Applied Pyrolysis, 21: 147-162.

Faix O, Schweers W. 1974. Vergleichende Untersuchungen an Polymermodellen des Lignins(DHP's) verschiedener Zusammensetzungen. 4. Mitteilung: UV-spektroskopische Untersuchungen. Holzforschung, 28: 94-98.

Falck R. 1912. Die Meruliusfaule des Bauholzes. Hausschwammforsch, 6: 1-405.

Fardim P, Gustafsson J, Schoultz SV, Peltonen J, Holmbom B. 2005. Extractives on fiber surfaces investigated by X.P.S. ToF-SIMS and AFM. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 255: 91-103.

Fergus BJ, Goring DAI. 1970a. The location of guaiacyl and syringl lignina in birch xylem tissue. Holzforschung, 24(4): 113-117.

Fergus BJ, Goring DAI. 1970b. The distribution of lignin in birch wood as determined by ultraviolet microscopy. Holzforschung, 24(4): 118-124.

Fergus BJ, Procter AR, Scott JAN, Goring DAI. 1969. The distribution of lignin in sprucewood as determined by ultraviolet microscopy. Wood Science and Technology, 3(2): 117-138.

Frisvad JC, Samson RA. 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of the food and airborne terverticillate penicillia and their mycotoxins. Studies in Mycology, 49: 1-173.

Gerlach D. 1977. Botanische Mikrotechnik. Georg Thieme Verlag Stuttgart, Germany.

Gersonde M, Becker G. 1958. Prüfung von Holzschutzmitteln für den Hochbau auf Wirksamkeit gegen Pilze an praxisgemäßen Holzproben ("Schwammkeller"-Versuch). Holz Roh- Werkstoff, 16: 346-357.

Grosser D. 1985. Pflanzliche und tierische Bau- und Werkholzschädlinge. DRW Weinbrenner, Lei-nfelden-Echterdingnen, Germany.

Grosser D, Liese W. 1971. On the anatomy of Asian bamboos, with special reference to their vascular bundles. Wood Science and Technology, 5(4): 290-312.

Guha SRD, Chandra A. 1979. Studies on the decay of bamboo *(Dendrocalamus strictus)* during outside storage - I. Effect of preservatives II. Effect on pulping qualities. The Indian Forester, 105(4): 293-300.

Hamaguchi T. 1953. The relation between the season of felling bamboo and its decay. Journal of Japanese Forestry Society, 35(3): 85-87.

Hamid NH, Abd Latif M, Sulaiman O. 2003. Decay resistance of bamboo (*Gigantochloa scortechinii*) compared to 24 Malaysian hardwood. XII. World Forestry Congress 2003, Québec, Canada, www.fao.org/docrep/article/wfc/XII/0039-b4.htm.

Hawksworth DL. 1991. The fungal dimension of biodiversity: Magnitude, significance, and conservation. Mycological Research, 95(6): 641-655.

Hawksworth DL. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological Research, 105(12): 1422-1432.

Hawksworth DL, Rossman AY. 1997. Where are all the undescribed fungi? Phytopathology, 87: 888-891.

Hawksworth DL, Crous PW, Redhead SA, et al. 2011. The Amsterdam Declaration on fungal nomenclature. IMA Fungus, 2(1): 105-112.

Hesse M, Meier H, Zeeh B. 1991. Spektroskopische Methoden in der organischen Chemie. Georg Thieme Verlag, Stuttgart, Germany.

Hibbert DS, Binder M, Bischoff JF, et al. 2007. A higher-level phylogenetic classification of the Fungi. Mycological Research, 111: 509-547.

Higuchi T. 1997. Chemistry and biochemistry of bamboo. Bamboo Journal, 4: 132-145.

Huckfeldt T, Schmidt O. 2006. Hausfaule- und Bauholzpilze. Rudolf Muller, Koln, Germany.

Hyde KD, Zhou D, Dalisay TE. 2002. Bambusicolous fungi: A review. Fungal Diversity, 9: 1-14.

Inari GN, Petrissans M, Lambert J, Ehrhardt JJ, Gerardin P. 2006. XPS characterization of wood chemical composition after heat-treatment. Surface and Interface Analysis, 38:1336-1342.

Jahn H. 1990. Pilze an Bäumen. Patzer, Berlin, Germany.

Jennings DH, Lysek G. 1999. Fungal biology, 2nd edn. Bios, Oxford, United Kingdom.

Jiang Z. 2007. Bamboo and rattan in the world. China Forestry Publishing House, Beijing, China.

Johansson LS, Campbell JM. 2004. Reproducible XPS on biopolymers: cellulose studies. Surface and Interface Analysis, 36: 1018-1022.

Kaune P. 1970. Bedingungen für das Prüfen mit Moderfäulepilzen im Vermiculit-Eingrabe-Verfahren. Material und Organismen, 5(2): 95-112.

Kendrick B. 1992. The fifth Kingdom, Mycologue Publications. Waterloo, Ontario, Canada.

Kim JJ, Lee SS, Ra JB, Lee H, Huh N, Kim GH. 2011. Fungi associated with bamboo and their decay capabilities. Holzforschung, 65(2): 271-275.

Kim JS, Lee KH, Cho CH, Koch G, Kim YS. 2008. Micromorphological characteristics and lignin distribution in bamboo (*Phyllostachys pubescens*) degraded by the white rot fungus *Lentinus edodes*. Holzforschung, 62(4): 481-487.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth & Bisby's Dictionary of the Fungi, ninth ed. CABI Publishing, Wallingford, United Kingdom.

Kirk PM, Cannon PF, Minter DW, Stalpers AJ. 2008. Dictionary of the Fungi, 10th ed. CABI, Wallingford, United Kingdom.

Kleist G, Morris I, Murphy R. 2002. Invasion and colonization of bamboo culm material by stain and decay fungi. International Research Group on Wood Preservation, Stockholm, Sweden, IRG/WP 02-10453.

Koch G, Kleist G. 2001. Application of scanning UV microspectrophotometry to localise lignins and phenolic extractives in plant cell walls. Holzforschung, 55(6): 563-567.

Kumar S, Schukla KS, Dev T, Dobriyal PB. 1994. Bamboo preservation techniques: a review INBAR Technical Report, No. 03.

Lee KH, Cho CH, Kim YS. 2006. Micromorphology of bamboo fibers degraded by brown-rot fungus Gloeophyllum trabeum. The International Research Group on Wood Preservation, Stockholm, Sweden, IRG/WP 06-10576.

Leithoff H, Peek R. 2001. Heat treatment of bamboo. The International Research Group on Wood Preservation, Stockholm, Sweden, IRG/WP 01-40216.

Leutritz J. 1946. A wood-soil contact culture technique for laboratory study of wood destroying fungi, wood decay and wood preservation. Bell System Technical Journal, 25(1): 102-135.

Leveille JH. 1845. Champignons exotiques. Annales des Sciences Naturelles Botanique, 3(3): 38-71.

Li K, Reeve DW. 2004. Sample contamination in analysis of wood pulp fibers with X-ray photoelectron spectroscopy, Journal of Wood Chemistry and Technology, 24:183-200.

Liese W.1959a. Bamboo preservation and soft-rot. Food and Agriculture Organization Report to the Government of India, FAO Document No. 1106.

Liese W. 1959b. Die Moderfäule, eine neun Krankheit des Holzes. Naturwiss Rundschau, 11: 419-425.

Liese W. 1970. Ultrastructural aspects of woody tissue disintegration. Annual Review of Phytopathology, 8: 231-258.

Liese W. 1985. Bamboos - biology, silvics, properties, utilization. Schriftenreihe Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany, SGTZ Document No. 180.

Liese W. 1998. The anatomy of Bamboo culms. International Network for Bamboo and Rattan, Beijing, China.

Liese W, Kumar S. 2003. Bamboo preservation compendium. Technical report 22. International Network for Bamboo and Rattan, Beijing, China.

Liese W, Schmidt R. 1966.Untersuchungen zum Zellwandabbauvon Nadelholz durch Trametes pini. Holz als Roh- und Werkstoff, 24(10): 454-460.

Lombard FF, Chamuris GP. 1990. Basidiomycetes. In: Wang CJK and Zabel RA (eds.) Identification manual for fungi from utility poles in the eastern United States. Am Type Culture Collection, Rockville, United States, pp 21-104.

Lybeer B, Koch G. 2005a. A topochemical and semiquantitative study of the lignification during ageing of bamboo culms (*Phyllostachys viridiglaucescens*). IAWA Journal, 26(1): 99-109.

Lybeer B, Koch G. 2005b. Lignin distribution in the tropical bamboo species *Gigantochloa levis*. IAWA Journal, 26(4): 443-456.

Lybeer B, Koch G, Van Acker J, Goetghebeur P. 2006. Lignification and cell wall thickening in nodes of *Phyllostachys viridiglaucescens* and *Phyllostachys nigra*. Annals of Botany, 97: 529-539.

Ma X, Jiang M. 2009. Isolation and identification of stain fungi and mould fungi on bamboo wood in china. International Research Group on Wood Protection, Stockholm, Sweden, IRG/WP 09-10703.

Ma X, Jiang M, Qin D. 2010. The invasion channels of damage fungi in bamboo lumber. International Research Group on Wood Protection, Stockholm, Sweden, IRG/WP 10-10712.

Mariey L, Signolle JP, Amiel C, Travert J. 2001. Discrimination, classification, identification of microorganisms using FTIR spectroscopy and chemometrics. Vibrational. Spectroscopy, 26: 151-159.

Mathias R, Koch G. 2011. Topochemical investigation of early stagess of lignin modification within individual cell wall layers of Scots pine (*Pinus sylvestris* L.) sapwood infected by the brown-rot fungus *Antrodia vaillantii* (DC.: Fr.) Ryv. International Biodeterioration & Biodegradation, 65: 913-920.

McLaughlin DJ, McLaughlin EG, Lemke PA. 2001a. The Mycota. Vol. VII. Part A. Systematics and Evolution. Springer, Berlin, Germany.

McLaughlin DJ, McLaughlin EG, Lemke PA. 2001b. The Mycota.Vol. VII. PartB. Systematics and Evolution. Springer, Berlin, Germany.

Mohanan C. 1997. Diseases of bamboos in Asia. International Development Research Centre, New Delhi, India.

Murphy RJ, Alvin KL, Tan YF. 1991. Development of soft rot decay in the bamboo *Sinobambusa tootsik*. IAWA Bulletin n s, 12(1): 85-94.

Musha Y, Goring DAI. 1975. Distribution of syringyl and guaiacyl moieties in hardwoods as indicated by ultraviolet microscopy. Wood and Science Technology, 9: 45-58.

Naumann A, Navarro-González M, Peddireddi S, Kües U, Polle A. 2005. Fourier transform infrared microscopy and imaging: Detection of fungi in wood. Fungal Genetics and Biology, 42(10): 829-835.

Naumann A, Polle A. 2006. FTIR imaging as a new tool for cell wall analysis of wood. New Zealand Journal of Forestry Science, 36(1): 54-59.

Naumann D, Helm D, Labischinski H. 1991. Microbiological characterizations by FT-IR spectroscopy. Nature, 351: 81-82.

Ngo-Thi NA, Kirschner C, Naumann D. 2003. Characterization and identification of microorganisms by FT-IR microspectrometry. Journal of Molecular Structure, 661-662: 371-380.

Nobles MK. 1965. Identification of cultures of wood-inhabiting Hymenomycetes. Canadian Journal of Botany, 43: 1097-1139.

Nzokou P, Kamdem DP. 2005. X-ray photoelectron spectroscopy study of red oak- (*Quercus rubra*), black cherry- (*Prunus serotina*) and red pine- (*Pinus resinosa*) extracted wood surfaces. Surface and Interface Analysis, 37:689-694.

Papadopoulos AN. 2010. Durability of particleboards made from wood particles chemically modified with propionic anhydride: results after six years in ground stake-test. European Journal of wood and wood products, 68(3):353-354.

Pawley JB. 2006. Handbook of Biological Confocal Microscopy (3rd ed.). Springer, Berlin, Germany.

Peylo A. 2001. Schnellanalyse von Holzschutzmitteln. Schützen & Erhalten September 2001, 25-27.

Purushotham A. 1963. Utilization of bamboo. Journal of the Timber Development Association of India, 9(2): 2-19.

Purvis A, Hector A. 2000. Getting the measure of biodiversity. Nature, 405: 212-219.

Raper KB, Fennell DI. 1965. The genus *Aspergillus*. Williams and Wilkins, Baltimore, United States.

Razak W, Hashim WS, Murphy RJ. 2002. SEM observation on the decay of bamboo *Gigantochloa scortechinii* exposed in tropical soil. Journal of Tropical Forest Products, 8(2): 168-178.

Razak W, Mahmud S, Hashim WS. 2005. Fungal colonisation and decay in tropical bamboo species. Journal of Applied Sciences, 5(5): 897-202.

Razak W, Mahmud S, Tamizi M, Awang AMY. 2006. Durability performance of *Gigantochloa scortechinii* through laboratory fungal decay tests. Research Journal of Microbiology, 1(2): 198-202.

Remadevi OK, Muthukrishnan R, Nagaveni HC, Sundararaj R, Vijayalakshmi G. 2005. Durability of bamboos in India against termites and fungi and chemical treatments for its enhancement. International Research Group on Wood Protection, Stockholm, Sweden, IRG/WP 05-10553.

Remadevi OK, Revathi TG, Jain SH, Joshi SC. 2012. Susceptibility of Bamboo species in India to the attack of powder post beetle, *Dinoderus minutus* and prophylactic and curative measures for their management. 9th World Bamboo Congress Proceedings, Antwerp, Belgium, 883-897.

Ryvarden L, Gilbertson RL. 1993. European polypores. Part1. Synopsis Fungorum 6, Fungi flora Oslo, Norway.

Ryvarden L, Gilbertson RL. 1994. European polypores. Part1. Synopsis Fungorum 7, Fungi flora Oslo, Norway.

Salzer R, Steiner G, Mantsch HH, Mansfield J. 2000. Infrared and Raman imaging of biological and biomimetic samples. Fresenius Journal of Analytical Chemistry, 366: 712-726.

Samson RA, Frisvad JC. 2004. *Penicillium* subgenus *Penicillium*: new taxonomic schemes, mycotoxins and other extrolites. Studies in Mycology, 49: 1-257.

Samson RA, Varga J. 2007. *Aspergillus* systematics in the genomic era. Studies in Mycology, 59: 1-206.

Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B. 2010. Food and indoor fungi. CBS laboratory manual series 2. Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Sarkanen KV, Hergert HL, 1971. Classification and distribution. In: Sarkanen, K.V.,Ludwig, C.H. (Eds.), Lignins, occurrence, formation, structure and reactions. Wiley Interscience, New York, United States, pp 43-49.

Savory JG. 1954. Damage of wood caused by micro-organisms. Journal of Applied Microbiology, 17(2): 213-218.

Schmidt O. 1986. Experiments with mushroom cultivation on wood waste. Plant Research and Development, 24: 85-92.

Schmidt O. 2006. Wood and tree fungi. Biology, damage, protection, and use. Springer, Berlin, Germany.

Schmidt O, Liese W. 1978. Biological variations within *Schizophyllum commune*. Material und Organismen, 13(3): 169-185.

Schmidt O, Liese W. 1980. Variability of wood degrading enzymes of *Schizophyllum commune*. Holzforschung, 34(2): 67-72.

Schmidt O, Grimm K, Moreth U. 2002. Molecular identity of species and isolates of the *Coniophora* cellar fungi. Holzforschung, 56(6): 563-571.

Schmidt O, Wei D, Liese W, Wollenberg E. 2011. Fungal degradation of bamboo samples. Holzforschung, 65(6): 883-888.

Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink M, Hill CF, Zalar P, De Hoog GS, Crous PW. 2007. Biodiversity in the *Cladosporium herbarum* complex (*Davidiellaceae*, *Capnodiales*), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. Studies in Mycology, 58: 105-156.

Scott JAN, Procter AR, Fergus BJ, Goring DAI. 1969. The application of ultraviolet microscopy to the distribution of lignin in wood description and validity of technique. Wood Science and Technology, 3(1): 73-92.

Singh A, Schmitt U, Möller R, Dawson B, Koch G. 2006. Ray tracheids in *Pinus radiata* are more highly resistant to soft rot as compared to axial tracheids: relationship to lignin concentration. Wood Science and Technology, 40:16-25.

Sinn G, Reiterer GS, Stanzl-Tschegg SE. 2001. Surface analysis of different wood species using X-ray photoelectron spectroscopy (XPS). Journal of Materials Chemistry, 36: 4673-4680.

Sinner M, Puls J. 1978. Non-corrosive dye reagent for detection of reducing sugars in borate complex ion-exchange chromatography. Journal of Chromatography, 156: 197-204.

Spurr AR. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research, 26: 31-43.

Stalpers J. 1978. Identification of wood-inhabiting Aphyllophorales in pure culture. Studies in Mycology, 16: 1-248.

Suhirman, Khusniati T. 1987. Laboratory study on the effect of mud-submersion treatment on the durability of two bamboo and one wood species against fungi. Material and Organisms, 22(4): 289-296.

Sulthoni AA. 1990. Simple and cheap method of bamboo preservation, Bamboos, Current research, Proceedings of the International Bamboo Workshop, Cochin, India, 14-18 Nov 1988. Kerala Forest Research Institute, Kerala, India, pp 209-211.

Sun F, Duan X. 2004. Situation and overview of bamboo mold-preservation, Word bamboo and rattan, 2(4): 1-4.

Sungkaew S, Stapleton CMA, Salamin N, Hodkinson TR. 2009. Non-monophyly of the woody bamboos (Bambuseae; Poaceae): a multi-gene region phylogenetic analysis of Bambusoideae s.s. Journal of Plant Research, 122: 95-108.

Suprapti S. 2010. Decay resistance of five Indonesian bamboo species against fungi. Journal of Tropical Forest Science, 22(3): 287-294.

Takabe K. 2002. Cell walls of woody plants: autoradiography and ultraviolet microscopy. In: Chaffey, N. (Ed.), Wood formation in trees. Taylor & Francis, London, United Kingdom, pp 159-177.

Tang TKH, Schmidt O, Liese W. 2009. Environment-friendly short-term protection of bamboo against moulding. Journal of the Timber Development Association of India, 55: 8-17.

Tang TKH, Schmidt O, Liese W. 2012. Protection of bamboo against mould using environment-friendly chemicals. Journal of Tropical Forest Science, 24(2): 285-290.

Varga J, Samson RA. 2008. *Aspergillus* in the genomic era. Wageningen Academic Publishers, Wageningen, the Netherlands.

Varma RV, Mathew G, Mohanadas K, Gnanaharan R, Nair KS. 1988. Laboratory evaluation of insecticides for the control of the bamboo borers. *Dinoderus minutus* and *D. ocellaris* (Coleoptera: Bostrychidae). Material und Organismen, 23(4): 281-288.

Vena FV, Brienzo M, Garcá-Aparicio M del P, Görgens JF, Rypstra T. 2013. Hemicelluloses extraction from giant bamboo (*Bambusa balcooa* Roxburgh) prior to kraft or soda-AQ pulping and its effect on pulp physical properties. Holzforschung, 67(8): 863-870.

Wang SF, Hsieh TC. 1968. Durability records of treated and untreated bamboo. Cooperative of Bulletin Taiwan Forestry Research Institute, Taipei, Taiwan, CBTFRI Working Paper No. 15.

Wang W, Hui C, Chen Y, Fu H. 2000. Mildew and rot of bamboo wood and mold fungi. Journal of Bamboo Research, 19(2):40-43.

Wang W, Hui C, Chen Y, Fu H, Yang Y. 2000. A study for mold and rot fungi. Journal of Bamboo Research, 19(4): 26-35.

Wang W, Hui C, Liu C, Chen Y, Fu H. 1999. A study on the chemical com positions of 14 timber bamboo species in Yunnan province. Journal of Bamboo Research, 18(2): 74-78.

Wei D, Schmidt O, Liese W. 2012. Susceptibility of bamboo to fungi. 9th World Bamboo Congress Proceedings, Antwerp, Belgium, pp 235-245.

Wen T, Chou W. 1984. A report on the anatomy of the vascular bundle of bamboos from China(1). Journal of Bamboo Research, 3: 1-21.

White TJ, Bruns TD, Lee S, Taylor JW, 1990. Amplification and direct sequencing of fungalribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky J, White TJ (eds), PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego, United States, pp 315-322.

Whittaker RH. 1969. New concepts of kingdoms of organisms. Science, 163: 150-160.

Willfö S, Pranovich A, Tamminen T, Puls J, Laine C, Suurnäkk A, Saake B, Uotila K, Simolin H, Hemming J, Holmbom B. 2009. Carbohydrate analysis of plant materials with uronic acid-containing polysaccharides–A comparison between different hydrolysis and subsequent chromatographic analytical techniques. Industrial Crops and Products, 29: 571-580.

Worrall JJ, Wang CJK. 1991. Importance and mobilization of nutrients in soft rot of wood. Canadian Journal of Microbiology, 37(11): 864-868.

Worrall JJ, Anagnost SE, Wang CJK. 1991. Conditions for soft rot of wood. Canadian Journal of Microbiology, 37(11): 869-874.

Wu G, Lin X, Ran L. 1994. Identification of moulds infecting bamboo wood and formulation of fungicidal compounds. Economic Forest Researches, 12(2): 50-55.

Wu K, Wen Y. 2000. Bamboo mildew-rotting and its relation with environmental condition. Forest Research, 13(1): 63-70.

Xu G, Wang L, Liu J, Wu J. 2013. FTIR and XPS analysis of the changes in bamboo chemical structure decayed by white-rot and brown-rot fungi. Applied Surface Science 280: 799-805.

Zalar P, De Hoog GS, Schroers H-J, Crous PW, Groenewald JZ and Gunde-Cimerman N. 2007. Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. Studies in Mycology, 58: 157-183.

Zhang X, Yu H, Huang H, Y Liu. 2007. Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culm. International Biodeterioration & Biodegradation, 60(3): 159-164.

Zhao G, He W, Jin Z. 1994. Studies on the fungi on the bamboo wood. Journal of Nanjing Forestry, 18(3): 87-90.

Zhou F. 1999. Looking back to bamboo industry for the 20th century, looking forward bamboo industry for the 21th century. Journal of Bamboo Research, 18(4): 1-10.

List of publications

Publication I

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Publication III

Wei D, Schmidt O, Liese W. 2013. Durability test of bamboo against fungi according to EN standards. European Journal of Wood and Wood Products, 71(5): 551-556.

Publication IV

Wei D, Schmidt O, Liese W. 2013. Method to test fungal degradation of bamboo and wood using vermiculite as reservoir for moisture and nutrients. Maderas. Ciencia y tecnolog á, 15(3): 349-356.

Conference contributions

Oral presentations

Wei D, Schmidt O, Liese W. Susceptibility of bamboo to fungi. 9th World Bamboo Congress, Antwerp, Belgium, 10 April, 2012.

Wei D. Pilzempfindlichkeit von Bambus. Wood Biology Colloquium, University of Hamburg, Germany, 03 May, 2012.

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Poster

Wei D, Schmidt O, Liese W. Molecular identification of bamboo-inhabiting and degrading fungi. Annual Conference of the Association for General and Applied Microbiology, Karlsruhe, Germany, 3-6 April, 2011.

Wei D, Schmidt O, Schmitt U, Liese W. Fungal decay studies on bamboo species. 4th Congress of European Microbiologists, Geneva, Switzerland, 26-30 June, 2011.

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Appendices

Publication I

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Fungal degradation of bamboo samples

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Abstract

The degradation of several Asian bamboo species by white-, brown-, and soft-rot fungi was investigated under laboratory conditions by means of different test methods. Severe deterioration was caused by all three fungi types. The bamboo species differed in durability. Samples from 6 months young culms decayed more than older ones. There were no significant differences between 1- and 3-year-old culms. Samples taken from the culm top were more vulnerable to decay than those from the bottom. Wet bamboo samples with soil contact were especially degraded by the white-rot fungus *Schizophyllum commune*, whereas the brown-rot fungus *Coniophora puteana* produced the greatest mass loss in drier samples. The sealing of bamboo crosscut ends reduced the rate of decay.

Keywords: bamboo; bamboo moisture content; brown-rot fungi; culm age; fungal degradation; soft-rot fungi; white-rot fungi.

Introduction

Bamboo is an abundant natural product with excellent technological properties. However, the use of bamboo is restricted due to its susceptibility to deteriorating organisms and its generally low durability (Liese and Kumar 2003). Several publications are available on the decay of bamboo by fungi and on its natural durability (Banerjee and Mukhopadhyay 1962; Purushotham 1963; Abdurachim 1964; Liese 1985; Murphy et al. 1991; Leithoff and Peek 2001; Hamid et al. 2003; Zhang et al. 2007; Kim et al. 2008, 2011; Suprapti 2010; Ma et al. 2010). Such information can improve protection measures and lead to better utilisation of bamboo. Many data are available from field tests (i.e., ''graveyard'' tests: Liese 1959; Abdurachim 1964; Wang and Hsieh 1968; Razak et al. 2002).

The large body of literature data is not always consistent because of the variability of the bamboo species and fungi tested. Moreover, it is probable that the results are influenced by sampling (the height of the culm, where the sample was taken), the cutting season, moisture content, and the age of the plants. The intention of the present paper is a contribution to this complex subject. The results obtained from a large number of bamboo species under well-defined sampling and test conditions will be described. Though data from field tests are very useful, data obtained from laboratory experiments under controlled conditions are more precise and have a better reproducibility. This approach was chosen for the presented results.

Materials and methods

Investigated fungi

The fungi in focus are listed in Table 1. The isolates are stored in our strain collection.

Degradation tests in preserving jars

Investigations were conducted on culm sections of *Bambusa maculata* and *Gigantochloa atroviolacea* from Indonesia and *Phyllostachys pubescens* from Germany. Bamboo specimens were obtained from CONBAM (Geilenkirchen, Germany) and the Bamboo Centre (Baden-Baden, Germany). The curcuma-test (Peylo 2001) was applied to ensure that the samples are not contaminated with boron compounds which are common for moulding prevention during storage. Samples (3×1 cm^2) were dried at 103°C, weighed, and autoclaved. Household preserving jars with a volume of 500 ml were autoclaved for 20 min and used as culture vessels (Schmidt 1986). Culture medium for the basidiomycetes and ascomycetes were 110 ml malt (2%)-agar (1.5%) and Abrams agar (Savory 1954), supplemented with 0.1% yeast extract as vitamin source, respectively. The jars were inoculated with fresh mycelial agar plugs (8 mm^2) and incubated at 21°C and 70% RH for 12 months.

Evaluation of fungal mass loss in all experiments was done according to EN 113 (1996). Minimum and maximum mass loss values, species, the special fungal strains tested, and the number of sample replicates are presented in the Tables.

Degradation tests in Kolle flasks

To test the influence of culm age and sample location, the species *Bambusa polymorpha*, *Dendrocalamus strictus, Melocanna bambusoides, Oxytenanthera nigro-ciliata*, and *Thyrsostachys oliveri* were collected at the Experimental Forests of the Forest Research Institute (Dehra Dun, India), and of the College of Forestry (Laguna), during consultancy trips by one of the authors (W. Liese) between 1960 and 1970. Specimens with a wall thickness of 5×2 cm were autoclaved for 15 min at 121°C. The experiments with five parallels were performed according to the Kolle flask-method with malt-agar for the basidiomycetes (Liese et al. 1935, EN 113 1996) and Abrams-agar for the soft-rot fungi. A number of experiments were conducted on samples with sealed crosscut ends (KAHAPON, Kurt Herberts Coating Company, Wuppertal, Germany). Kolle flasks

Table 1 Fungi investigated.

Species	Type of rot	Codings for Lab. isolate	Codings for EN	Origin and isolation by
Pleurotus ostreatus	White	11	ATCC 44737	Tree, Steinach, Germany, W. Luthardt ≈1949
Schizophyllum commune	White	3		Wood, Spain, 1966
Schizophyllum commune	White	4		Philippines, 1966
Schizophyllum commune	White	87		*Dendrocalamus brandisii*, Costa Rica, D.S. Wei 2009
Schizophyllum commune	White	98		*Dendrocalamus asper*, Thailand, D.S. Wei 2009
Trametes versicolor	White	63	CTB 863A, EN 113	France
Coniophora puteana	Brown	1	Ebw. 15, EN 113	Wood, Berlin, J. Liese 1930
Coniophora puteana	Brown	167		Wood, Hamburg, O. Schmidt 1997
Gloeophyllum trabeum	Brown	183	Ebw. 109, EN 113	Wood, Eberswalde, Germany
Oligoporus placenta	Brown	120	FPRL 280, EN113	Wood, Berlin, J. Liese 1938
Chaetomium globosum	Soft	10	ATCC 44753	Wood, München 1963
Chaetomium globosum	Soft	76	ATCC 6205, EN 807	Wood, Berlin, BAM 1989
Paecilomyces variotii	Soft	13	ATCC 44741	Sugar cane bagasse, Trinidad, K. Walter 1977
Paecilomyces variotii	Soft	92	DSM 1961	Wood, Berlin, BAM 1990

were kept at 20°C for the basidiomycetes and at 28°C for the soft-rot fungi in a period of 4–6 months.

Degradation tests on samples with larger dimension (Fungus cellar-test)

Culm parts of *Arundinaria amabilis* (Vietnam), *Bambusa maculata* (Indonesia), *Dendrocalamus asper* (Indonesia), *Gigantochloa atroviolacea* (Indonesia), *Phyllostachys nigra* (Japan), *Phyllostachys nigra* 'Boryana' (China) and *Phyllostachys pubescens* (Germany) were obtained from the CONBAM company and the Bamboo Centre (see above). Sections (25 cm) were longitudinally halved, dried at 103°C and weighed, dipped in tap water for two days and autoclaved at 121°C for 40 min. Metal tubs (120 cm long, 60 cm wide) were filled with 30 l of unsterile compost soil from the institute garden (Gersonde and Becker 1958). Samples were placed either on autoclaved wood supports or directly on the soil. Each sample was infected with three small inoculation wood pieces (0.5 cm^3) containing mycelium. The tubs were covered with panes of glass and kept at 23°C and 90% RH for 1 year. The soil was moistened weekly with sprayed tap water.

Results and discussion

The fungi investigated (Table 1) are not endemic in the natural distribution area of bamboo. However, several strains served for decades in wood degradation experiments (Liese et al. 1935), which led to the German standard DIN 52 176 and to the current European standard EN 113 (1996). Some fungi occur on bamboo (e.g., Mohanan 1997) or have been isolated from bamboo culms in their natural habitat by the authors of the present study.

Table 2 Decay of bamboo species (min–max and average) expressed as % mass loss of three replicates after 1 year of incubation in preserving jars.

Fungus, code	Mass loss (%) caused on the species		
	Bambusa maculata	*Gigantochloa atroviolacea*	*Phyllostachys pubescens*
Pleurotus ostreatus 11	22.9–35.7	8.5–13.6	19.7–22.3
	28.2	10.6	21.0
Schizophyllum commune 87	2.7–2.9	6.7–6.8	4.7–5.6
	2.8	6.7	5.2
Schizophyllum commune 98	1.6–2.0	4.5–7.3	3.6–5.1
	1.8	5.6	4.4
Trametes versicolor 63	60.2–64.0	45.7–55.4	44.5–54.0
	62.5	51.6	47.8
Coniophora puteana 167	2.8–4.2	5.2–5.9	4.6–4.8
	3.6	5.6	4.7
Gloeophyllum trabeum 183	1.8–2.0	4.8–6.7	4.3–7.2
	1.9	5.7	5.3
Chaetomium globosum 10	31.2–32.7	7.6–12.7	36.9–39.7
	31.8	9.4	38.0
Paecilomyces variotii 13	0.8–1.4	3.3–4.0	3.4–4.2
	1.2	3.6	3.9

Table 2 shows the results of bamboo degradation after 1 year of incubation in preserving jars. Among the white-rot fungi, *Schizophyllum commune* caused the least decay (max 6.7% mass loss, ML), *Pleurotus ostreatus* medium decay (max 28.2% ML), and *Trametes versicolor* the highest (max 62.5% ML) decay. Abdurachim (1964) reported a 15% ML by *S. commune*. A low decay rate by *S. commune* was found by Suprapti (2010) and Kim et al. (2011) and was also reported for wood samples (Schmidt and Liese 1978). However, this species is commonly found on bamboo culms during storage and use (Liese 1985; Mohanan 1997; Liese and Kumar 2003). Kleist et al. (2002) showed that *S. commune* was the most successful coloniser among some fungi as it could penetrate bamboo via outer and inner culm walls, through cross section planes as well as through nodal ridges and longitudinally through nodiums. Both brown-rot fungi, *Coniophora puteana* and *Gloeophyllum trabeum*, were relatively inactive (causing max 5.7% ML). This finding is in contrast to observations made by Lee et al. (2006), who reported a 25% ML by *G. trabeum* in *Phyllostachys pubescens*, and by Ma et al. (2010), who found a 35% ML in *P. edulis*. Among the soft-rot fungi, *Paecilomyces variotii* was less aggressive, whereas *Chaetomium globosum* produced severe degradation, which also occurred in the other tests. A Japanese isolate of *Ch. globosum* caused less decay (Suprapti 2010). Experiments by Leithoff and Peek (2001) on *P. pubescens* and *P. virideglaucescens* in Petri dishes also gave comparable results with moderate decay by *S. commune* and *C. puteana* and stronger decay by soft-rot according to the standard ENV 807 (2001). Zhang et al. (2007) investigated 34 white-rot fungi and observed up to 15% ML in *P. pubescens; Pleurotus ostreatus* caused 5.2% and *Trametes versicolor* 13.6% ML. The white-rot fungus *Lentinula edodes* produced a 13% ML in *P. pubescens* (Kim et al. 2008). Suprapti (2010) observed intense decay by the white-rot fungus *Pycnoporus sanguineus* and the brown-rot species *Tyromyces palustris*. Kim et al. (2011) observed a ML of up to 22% by six white-rot fungi in samples of three *Phyllostachys* species and a maximum of 18% by 12 soft-rot fungi.

The tested bamboo species differed in susceptibility to fungi (see also Tables 4 and 5). However, more comparative studies are necessary. Suprapti (2010) showed *Bambusa vulgaris*, *Gigantochloa apus* and *G. atroviolacea* to be moderately resistant, whereas *G. pseudoarundinacea* and *Dendrocalamus asper* were not resistant. Hamid et al. (2003) found *G. scortechinii* less susceptible to brown-rot (*Coniophora puteana*) compared to white-rot (*Trametes versicolor*). Differences obtained by field tests have been also reported. *Phyllostachys makinoi* and *Ph. edulis* were more resistant to sapstain and decay fungi than *Leba dolicochoclada* and especially *Sinocalamus latiflorus* (Wang and Hsieh 1968).

It is likely that the cutting season has an influence on decay resistance because starch and protein content vary during the year and change with culm age (Magel et al. 2006). Hamaguchi (1953) investigated the decay resistance of three *Phyllostachys* species at monthly intervals and obtained the lowest mass loss in samples cut between September and February, the time after sprouting.

When comparing the results of mass loss experiments in the literature, the possibility of chemical pretreatment of bamboo species against fungi should be kept in mind. For example, boron treatment is often used before shipment to Europe and would severely affect subsequent decay experiments. Our samples were boron-free as proven by the curcuma-test. Furthermore, the results of all experiments on fungal activity strongly depend on the type of isolate involved; strain variation is common among fungi (Schmidt and Liese 1980). In our tests, Tables 2 and 3 show similar results for the two *S. commune* isolates.

The influence of culm age on decay resistance was investigated for *Melocanna bambusoides* (Table 3). Samples from the youngest culm (6 months) were more susceptible to fungi, particularly to *Trametes versicolor* and the two soft-rot fungi. The decay of samples from 1-, 2-, and 3-year-old

Table 3 Influence of culm age on decay of *Melocanna bambusoides* samples (min–max and average) expressed as % mass loss of five replicates after 6 months of incubation in Kolle flasks; unpublished data from 1970.

Fungus, code	Mass loss (%) as a function of the culm age in years			
	0.5 years	1 year	2 years	3 years
Schizophyllum commune 3	7.1–7.8	6.1–7.6	4.8–5.3	4.1–5.1
	7.4	6.9	5.0	4.6
Schizophyllum commune 4	8.1–10.2	5.8–7.0	5.1–5.8	3.1–5.0
	9.1	6.4	5.6	4.8
Trametes versicolor 63	21.6–42.6	15.8–17.8	11.5–13.4	12.7–16.9
	28.3	16.9	12.5	14.7
Coniophora puteana 1	8.5–15.3	10.3–17.0	6.8–9.5	6.3–12.8
	11.2	13.7	8.4	9.9
Oligoporus placenta 120	4.3–7.0	4.9–11.2	4.8–6.1	4.6–9.9
	5.6	6.8	5.4	6.0
Chaetomium globosum 76	51.2–54.5	30.2–32.8	31.1–34.7	25.4–30.4
	52.7	31.4	32.3	27.9
Paecilomyces variotii 92	18.1–20.2	8.4–10.7	6.2–10.3	6.6–10.4
	19.7	9.6	8.2	7.5

Table 4 Influence of culm section (top/base) and sample accessibility (all sample sides open/crosscut areas sealed) on decay (min–max and average) expressed as % mass loss of 5 replicates after 6 months of incubation in Kolle flasks; unpublished data from 1970.

			Mass loss (%) caused by the fungi				
Bamboo species	Culm section	Sample sealing	*Schizophyllum commune* 4	*Coniophora puteana* 1	*Oligoporus placenta* 120	*Chaetomium globosum* 76	*Paecilomyces variotii* 92
Bambusa polymorpha	Top	No	4.6–6.1	11.5–14.4	7.2–13.4	24.3–41.0	12.0–16.3
			5.9	12.1	12.8	33.7	14.1
		Yes	4.0–4.6	9.4–13.8	4.0–4.6	11.9–14.5	8.6–10.5
			4.4	12.4	4.4	13.3	9.7
	Base	No	3.6–4.3	6.2–16.3	3.1–5.7	20.7–26.6	7.9–10.2
			4.1	15.5	5.7	23.3	9.4
		Yes	2.8–3.7	4.4–6.2	1.4–2.3	11.7–14.2	5.8–9.9
			3.3	5.5	1.9	12.6	8.1
Dendrocalamus strictus	Top	No	2.5–3.0	22.9–45.9	5.7–13.6	21.6–33.5	6.8–11.7
			2.7	38.2	11.3	28.2	9.3
		Yes	1.8–3.0	6.9–11.3	3.6–12.6	13.5–19.3	3.2–5.2
			2.4	8.8	7.7	15.9	4.1
	Base	No	0.1–10.9	8.3–18.7	3.5–11.7	6.2–7.3	1.8–2.3
			4.7	12.7	9.8	6.7	2.1
		Yes	1.4–3.5	1.5–3.6	1.1–7.9	6.0–8.0	1.6–2.0
			2.0	2.7	3.0	7.2	1.8
Oxytenanthera nigro-ciliata	Top	No	0.7–3.0	26.8–51.0	0.7–17.5	38.5–43.6	1.7–17.5
			2.1	38.5	7.4	41.1	7.4
		Yes	1.8–2.7	8.9–15.1	0.2–4.1	8.2–13.8	1.6–3.6
			2.2	11.8	1.4	11.1	2.2
	Base	No	2.6–3.1	3.2–5.4	1.3–1.9	19.2–22.8	4.5–7.1
			2.8	4.0	1.6	21.1	6.0
		Yes	1.8–2.7	2.3–2.8	0.5–1.3	5.8–7.5	3.1–3.8
			2.2	2.6	0.8	6.5	3.5
Thyrsostachys oliveri	Top	No	5.2–8.5	11.9–26.9	3.4–8.9	38.7–54.2	6.5–8.9
			6.8	18.5	6.4	47.2	7.7
		Yes	6.0–7.7	9.5–11.5	4.8–6.4	13.0–13.7	7.8–9.5
			7.0	10.4	5.7	13.3	8.5
	Base	No	2.7–3.6	5.2–10.0	1.8–2.8	23.0–29.3	4.7–6.4
			3.2	8.2	2.3	27.2	5.2
		Yes	0.4–2.6	4.7–5.7	1.5–1.8	8.5–9.1	2.4–3.1
			1.9	5.3	1.6	8.8	2.7

culms differed only minimally. In *Gigantochloa scortechinii*, younger culms (6 months) were more susceptible to *Coniophora puteana* and *T. versicolor* than 6.5-year-old culms (Hamid et al. 2003). The higher decay rate in young culms can be attributed to a higher content of carbohydrates and proteins in parenchyma cells. On the other hand, Schwarze (2007) observed negligible degradation of balsa wood consisting of 92% parenchyma cells and explained this finding by inhibiting effect of parenchyma cells against brown-rot decay.

To investigate the influence of sample location within a culm, samples were taken from the top and bottom of a culm (Table 4). Half of the samples had sealed ends because bamboo cross ends have large vessels and are easily accessible for penetrating hyphae and therefore this fact may have an influence on decay. In general, samples from the top were more susceptible to decay than from the bottom. Top culms have the highest starch content (Abd Latif et al. 1992; Liese and Abd Latif 2000; Okahisa et al. 2007). Suprapti (2010) reported moderate resistance for the middle portion of a culm, whereas the bottom and top parts were not resistant. Sealing the ends may force the hyphae to penetrate through the epidermis of the samples and not through the vessels at the crosscut plane. In most cases, samples accessible from all sides were more decayed than sealed ones (Table 4). Kleist et al. (2002) also found the transverse faces to be the easiest route for fungi penetration into the culm.

To investigate less active fungi, e.g., *S. commune* and *C. puteana*, in more detail and to imitate natural conditions, decay tests with bigger sized samples were performed in the 'Fungus cellar' by incubating bamboo samples in unsterile compost soil in large metal tubs (Table 5). Due to the not absolutely identical conditions in the two tubs, the Table shows the data of all samples. In contrast to results with small samples in the jar test, bamboo samples in the Fungus cellar were considerably more degraded by *C. puteana* (max 43%) and *S. commune* (max 20%). Obviously, the moisture conditions in the Fungus cellar-test influenced the activity of both fungi, whereby the white-rot fungus *S. commune* differed considerably from the brown-rot species *C. puteana.*

Table 5 Decay (% mass loss, ML) and final moisture content (MC, %) of bamboo samples in metal tubs after 1 year in fungus cellar-test.

Species	Tub	Soil contact	*Coniophora puteana* 167		*Schizophyllum commune* 87	
			ML (%)	MC (%)	ML (%)	MC (%)
Arundinaria amabilis	1	Yes	15.5	187	15.3	148
	1	No	38.6	41	10.8	31
	2	No	43.4	57	8.7	68
	2	No	39.0	56	7.1	40
Bambusa maculata	1	Yes	9.9	159	11.0	174
	1	No	20.4	39	5.1	28
	2	No	38.3	48	5.0	33
	2	No	34.3	52	3.6	37
Dendrocalamus asper	1	Yes	5.4	104	5.1	90
	1	No	29.3	42	4.3	28
	2	No	26.9	45	3.8	32
	2	No	28.5	50	4.3	31
Gigantochloa atroviolacea	1	Yes	6.1	95	6.1	95
	1	No	18.7	34	4.7	25
	2	No	41.6	58	3.0	38
	2	No	42.9	57	5.0	43
Phyllostachys nigra	1	Yes	9.7	112	16.4	126
	1	No	32.6	49	9.1	32
	2	No	39.7	65	6.6	40
	2	No	40.7	56	6.4	52
Phyllostachys nigra 'Boryana'	1	Yes	35.3	103	19.7	182
	1	No	38.8	54	6.7	26
	2	No	37.9	60	5.7	40
	2	No	38.8	46	5.1	35
Phyllostachys pubescens	1	Yes	6.3	61	6.3	63
	1	No	5.4	32	5.4	24
	2	No	38.3	42	5.7	31
	2	No	31.2	43	6.3	36

Schizophyllum commune decayed samples with soil contact and thus with high water content (90–182% MC) more than samples without soil contact. In contrast, *C. puteana* produced a higher mass loss in samples located on wood supports and with a lower water content (34–65% MC). For practical purposes, bamboo samples in the range of 24–182% MC were attacked by basidiomycetes. However, due to the unsterile soil in the Fungus cellar, samples became contaminated by moulds which may also have excreted a growth promoting substance for *S. commune*. Substances from the soil or vitamins from soil bacteria may have also affected the fungus.

When comparing the decay resistance of bamboo in comparison to that of wood, the different anatomical and chemical composition of both tissues must be taken into account. Whereas wood contains about 80–95% sclerenchyma cells in form of fibres and tracheids, the amount of these types of cells is in bamboo much lower. The culm consists of roughly 50% parenchyma cells, 40% fibres and 10% conducting cells (vessels and phloem; Grosser and Liese 1971; Liese 1998). These proportions tend to vary greatly within the culm; at the culm base, the quantity of parenchyma cells is highest and decreases with increasing height with a corresponding increase in the proportion of fibres. Field tests with *Gigantochloa apus* by Abdurachim (1964) showed a similar low durability of this bamboo and the wood of *Hevea brasiliensis*. Hamid et al. (2003) classified the resistance of *Gigantochloa scortechinii* against brown-rot and white-rot among 24 Malaysian heavy and medium hardwoods. When comparing *Dendrocalamus asper*, *D. gigantochloa* and *Albizia falcata*, Suhirman and Khusniati (1987) attributed the low mass loss of bamboo species (18–21%) to their relatively high lignin content (24–27%). After six weeks, our decay tests comparing bamboo and wood revealed a 25% ML by *Chaetomium globosum* in *Oxythenanthera abyssinica* and 45% ML in the non-resistant hardwood beech.

In summary, the results show that all bamboo species investigated in this study can be considerably degraded by white-, brown- and soft-rot fungi with distinct differences between species, culm location and sample moisture content.

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References

Abd Latif, M., Khoo, K.C., Nor Azah Mohd, A. (1992) Carbohydrates in some natural stand bamboos. J. Tropic. For. Sci. 4:310–316.

Abdurachim, M.R.A. (1964) Bamboo preservation in Indonesia. Rimba Indonesia 9:66–76.

Banerjee, S., Mukhopadhyay, S.A. (1962) A study on *Merulius similis* B. & FR. and the associated bamboo rot. Oester. Bot. Zeitschr. 109:197–212.

EN 113 (1996) Determination of the toxic values of wood preservatives against wood destroying Basidiomycetes cultured on agar medium. Beuth, Berlin, 15 pp.

ENV 807 (2001) Wood preservatives – Determination of the effectiveness against soft rotting micro- and other soil inhabiting micro-organisms. Beuth, Berlin, 40 pp.

Gersonde, M., Becker, G. (1958) Prüfung von Holzschutzmitteln für den Hochbau auf Wirksamkeit gegen Pilze an praxisgemäßen Holzproben (''Schwammkeller''-Versuch). Holz Roh- Werkst. 16:346–357.

Grosser, D., Liese, W. (1971) On the anatomy of Asian bamboos, with special reference to their vascular bundles. Wood Sci. Technol. 5:290–312.

Hamaguchi, T. (1953). The relation between the season of felling bamboo and its decay. J. Jap. For. Soc. 35:85–87.

Hamid, N.H., Abd Latif, M., Sulaiman, O. (2003) Decay resistance of bamboo (*Gigantochloa scortechinii*) compared to 24 Malaysian hardwood. XII. World Forestry Congr. 2003, Québec, Canada, www.fao.org.docrep/article/wfc/XII/0039-b4.htm.

Kim, J.S., Lee, K.H., Cho, C.H., Koch, G., Kim, Y.S. (2008) Micromorphological characteristics and lignin distribution in bamboo (*Phyllostachys pubescens*) degraded by the white rot fungus *Lentinus edodes*. Holzforschung 62:481–487.

Kim, J.J., Lee, S.-S., Ra, J.-B., Lee, H., Huh, N., Kim, G.-H. (2011) Fungi associated with bamboo and their decay capabilities. Holzforschung 65:271–275.

Kleist, G., Morris, I., Murphy, R. (2002) Invasion and colonization of bamboo culm material by stain and decay fungi. Int. Res. Group Wood Preserv. IRG/WP/02-10453, 10 pp.

Lee, K.H., Cho, C.H., Kim, Y.S. (2006) Micromorphology of bamboo fibers degraded by brown-rot fungus *Gloeophyllum trabeum*. Int. Res. Group Wood Preserv. IRG/WP/06-10576, 9 pp.

Leithoff, H., Peek, R. (2001) Heat treatment of bamboo. Int. Res. Group Wood Preserv. IRG/WP/01-40216, 11 pp.

Liese, J., Nowak, A., Peters, F., Rabanus, A., Krieg, W., Pflug, H. (1935) Toximetrische Bestimmung von Holzkonservierungsmitteln, Beiheft 11 Angew. Chemie, Chem. Fabrik, 18 pp.

Liese, W. (1959) Bamboo preservation and soft-rot. FAO Report to the Government of India 1106, 36 pp.

Liese, W. (1985) Bamboos – biology, silvics, properties, utilization. Schriftenreihe GTZ, Eschborn, 180, 132 pp.

Liese, W. (1998) The anatomy of bamboo culms. INBAR TR 18, Beijing, China, 204 pp.

Liese, W., Abd Latif, M. (2000) The starch content of two Malaysian bamboos in relation to age, culm height, site and harvesting month. XII IUFRO World Congress, Poster, Vol. 3, 261.

Liese, W., Kumar, S. (2003) Bamboo preservation compendium. INBAR, Beijing, China, Technical Rep. 22, 231 pp.

Ma, X., Jiang, M., Qin, D. (2010) The invasion channels of damage fungi in bamboo lumber. Int. Res. Group Wood Preserv. IRG/WP 10-10712, 7 pp.

Magel, E., Kruse, S., Lütje, G., Liese, W. (2006) Soluble carbohydrates and acid invertases involved in the rapid growth of developing culms in *Sasa palmata* (Bean) Camus. Bamboo Sci. Cult. 19:23–29.

Mohanan, C. (1997) Diseases of bamboos in Asia. Intern. Dev. Res. Centre, New Delhi, India. 228 pp.

Murphy, R.J., Alvin, K.L., Tan, Y.-F. (1991). Development of soft rot decay in the bamboo *Sinobambusa tootsik*. IAWA Bull. n. s. 12:85–94.

Okahisa, Y., Yoshimura, T., Sugiyama, J., Horikawa, E.Y., Imamura, Y. (2007) Longitudinal and radial distribution of free glucose and starch in moso bamboo (*Phyllostachys pubescens* Mazel). J. Bamboo Rattan 6:21–31.

Peylo, A. (2001) Schnellanalyse von Holzschutzmitteln. Schützen & Erhalten September 2001:25–27.

Purushotham, A. (1963) Utilization of bamboo. J. Timber Dev. Pres. Ass. 92:2–19.

Razak, W., Hashim, W.S., Murphy, R.J. (2002) SEM observation on the decay of bamboo *Gigantochloa scortechinii* exposed in tropical soil. J. Trop. For. Prod. 8:168–178.

Savory, J.G. (1954) Damage of wood caused by micro-organisms. J. Appl. Microbiol. 17:213–218.

Schmidt, O. (1986) Experiments with mushroom cultivation on wood waste. Plant Res. Dev. 24:85–92.

Schmidt, O., Liese, W. (1978) Biological variations within *Schizophyllum commune*. Materials Organisms 11:215–230.

Schmidt, O., Liese, W. (1980) Variability of wood degrading enzymes of *Schizophyllum commune*. Holzforschung 34:67–72.

Schwarze, F.W.M.R. (2007) Wood decay under the microscope. Fungal Biol. Rev. 21:133–170.

Suhirman, Khusniati, T. (1987). Laboratory study on the effect of mud-submersion treatment on the durability of two bamboo and one wood species against fungi. Materials Organisms 22: 289–296.

Suprapti, S. (2010) Decay resistance of five Indonesian bamboo species against fungi. J. Trop. For. Sci. 22:287–294.

Wang, S.-F., Hsieh, R.-Ch. (1968). Durability records of treated and untreated bamboo. Coop. Bull. Taiwan For. Res. Inst. 15, 26 pp.

Zhang, X., Yu, H., Huang, H., Liu, Y. (2007). Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culm. Int. Biodeter. Biodegr. 60:159–164.

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Publication II

Susceptibility of Bamboo to Fungi

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Abstract

Moulds and basidiomycetes were isolated from culms of several bamboo species from various countries and identified by DNA sequencing. Laboratory staining experiments of samples with pure cultures of blue-stain fungi revealed the colonization of the tissue with thick, brown hyphae, chlamydospores and penetration of lignified cell walls by transpressoria as it occurs also in wood cells. Degradation experiments with pure cultures of white -, brown - and soft- rot fungi using different test arrangements showed considerable mass loss particularly by white and soft rot. Micromorphology of fungal attack was demonstrated by transmission electron microscopy. Results improve the basic view on fungal damage of bamboo, which is needed for better utilization and suitable protection measures.

Keywords

Biodeterioration, Moulds, Blue Stain Fungi, Decay Fungi, Molecular Identification of Fungi, Micromorphology of Degradation

Introduction

Bamboo is an abundant natural product with excellent technological properties. However, the use of bamboo is restricted due to its susceptibility to deteriorating organisms and its low durability in general (Liese and Kumar 2003). Bamboo is rapidly colonized by moulds and blue stain fungi, particularly during overseas transport (Thang et al. 2009). Several publications deal with fungal decay of bamboo (Liese 1959, 1985; Banerjee and Mukhopathya 1962; Purushotham 1963; Abdurachim 1964; Wang and Hsieh 1968; Murphy et al. 1991; Razak et al. 2002; Hamid et al. 2003; Zhang et al. 2007; Schmidt et al. 2011). Micromorphology of fungal cell wall attack was reported for the white-rot mushroom *Lentinula edodes* (Kim et al. 2008) and the brown-rot fungus *Gloeophyllum trabeum* (Cho et al. 2008). More information will improve protection measures and lead to better utilization of bamboo. This paper gives an overview on laboratory experiments under controlled conditions.

Material and Methods

Fungal Isolation and Identification

Samples of bamboo culms were kindly provided by several colleagues in various countries and were sent to Hamburg by post. In most cases, culms hand been in practical use so that the species was unknown. Some Thailand samples belong to *Bambus multiplex* and *Dendrocalamus asper*. *D. brandisii* was obtained from Costa Rica and *Phyllostachys glaucoviridescens* from Germany (see Table 1). Fungi were isolated by transfer of mycelia or spores from infected regions of the culm surface on malt extract (Oxoid 2%) agar (Oxoid 1.5%) plates and were brought to pure cultures by subsequent subculturing at room temperature. Molecular identification was mainly according to Schmidt et al. (2002). Briefly, DNA from mycelia and spores was extracted with the DNeasy Plant Extraction Kit (Qiagen). PCR program with the Qiagen *Taq* Core Kit and the ITS1/ITS4 primer of White et al. (1990) in the MJ Research thermocycler was 4 min at 98°C, 35 cycles of 30 sec at 94°C, 30 sec at 54°C and 45 sec at 72°C followed by 7 min at 72°C. Suitable PCR products after electrophoresis in 2% agarose gels (Biozym DNA agarose in TAE buffer) with the Mupid-ex system (Advance, Tokyo) were purified with the QIAprep Spin Miniprep Kit (Qiagen) and were sequenced by Eurofins MWG Operon. Species identification was performed via sequence comparison with the DNA data bases by BLAST (see Table 1).

Blue-stain Tests

The blue-stain fungi *Alternaria tenuissima*, *Alternaria alternata*, *Botryosphaeria subglobosa*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Pestalotiopsis microspora* and *Phoma macrostoma* deriving from bamboo were used for staining experiments, together with *Hormonema dematioides* and *Aureobasidium pullulans* from the Institute strain collection. Autoclaved bamboo samples placed on malt agar plates were inoculated with mycelial plugs from precultures and incubated at room temperature. After culture, small pieces from discoloured samples were embedded in PEG and cut to 10 μm sections with a microtome and visualized by light microscopy.

Degradation Tests

Degradation tests were done using culm sections of *Bambusa maculata, Gigantochloa atroviolacea* and *Phyllostachys pubescens*. Sample (3 x 1 cm) preparation and mass loss evaluation followed EN 113 (1996). Household preserving jars (500 ml) with 110 ml malt-agar were used as culture vessels for

the basidiomycetes and Abrams agar (Savory 1954) for the soft-rot fungi. Fungi used were derived from the laboratory strain collection and *Schizophyllum commune* from bamboo (see Table 1)

To investigate less active fungi, e.g. *S. commune* and *C. puteana*, in more detail and to imitate more natural conditions, degradation tests were also performed with bigger bamboo samples, which partly were placed on unsterile soil (`Fungus cellar-test´). Culm parts (25 cm) of *Arundinaria amabilis* (Vietnam), *Bambusa maculata* (Indonesia), *Dendrocalamus asper* (Indonesia), *Gigantochloa atroviolacea* (Indonesia), *Phyllostachys nigra* (Japan), *Phyllostachys nigra* `Boryana´ (China) and *Phyllostachys pubescens* (Germany) were longitudinally halved, dried at 103°C and weighed, dipped in tap water for two days und autoclaved at 121°C for 40 min. Metal tubs (120 cm long, 60 cm wide) were filled with 30 litres of unsterile compost soil from the institute garden. Samples were placed either on autoclaved wood supports or directly on the soil. The tubs were covered with panes of glass and the soil moistened weekly with sprayed tap water (see Figure 5).

Transmission Electron Microscopy of Decayed Bamboo Samples

Micromorphology of fungal degrading pattern of bamboo cell walls was examined by transmission electron microscopy (Kim et al. 2008)). Briefly, small pieces from the incubated samples were fixed in a mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2), dehydrated in a graded acetone series and embedded in Spurr´s epoxy resin (Spurr 1969). The embedded tissue was serially sectioned on an ultramicrotome (RMC, MTX) with a diamond knife. Ultrathin sections (100 nm in thickness) were examined with a Jeol 1200 transmission electron microscope at an accelerating voltage of 80 kV after staining with 0.5% (w/v) potassium permanganate (prepared in citrate buffer) to contrast the lignin component. Some sections were stained also with uranyl acetate and citrate.

Results

Identification of Fungi Colonizing Used Bamboo

Table 1 shows the fungi isolated from bamboo culms which had been in use in various countries.

Table 1. Fungi isolated from bamboo and identified by their rDNA-ITS sequence

Bamboo Origin	Deuteromycetes/Ascomycetes (number of strains)	Basidiomycetes (number of strains)
Ethiopia		*Schizophyllum commune* (1)
China	*Alternaria alternata* (1) *A. tenuissima* (1) *Arthrinium phaeospermum* (1) *Cladosporium cladosporioides* (2) *Dothiorella gregaria* (1) *Fusarium asiaticum* (1) *F. culmorum* (1) *F. zeae* (1) *Nigrospora oryzae* (4) *Penicillium commune* (1) *P. chrysogenum* (1) *P. tricolor* (1) *P. variabile* (1) *Phoma macrostoma* (1)	
Costa Rica		*Schizophyllum commune* (1)
Germany	*Trichoderma koningiopsis* (2) *T. viride* (3)	
Indonesia		*Cyathus stercoreus* (1)
Philippines	*Penicillium citrinum* (1) *P. sumatraense* (1)	
Thailand	*Aspergillus nomius* (1) *A. repens* (1) *Botryosphaeria subglobosa* (1) *Cladosporium cladosporioides* (2) *Epicoccum nigrum* (2) *Penicillium brevicompactum* (1) *P. citrinum* (2) *P. pinophilum* (1) *Trichoderma atroviride* (1) *T. koningiopsis* (1)	*Schizophyllum commune* (6)
Vietnam	*Apiospora montagnei(2)* *Arthrinium phaeospermum* (1) *A. sacchari* (3) *Aspergillus flavus* (5) *A. niger* (2) *Botryosphaeria subglobosa* (5) *Epicoccum nigrum* (4) *Penicillium bialowiezense* (1) *P. biourgeianum* (1) *P. brevicompactum* (2) *P. expansum* (1) *P. islandicum* (1) *Pestalotiopsis microspora* (1)	
Number of fungal isolates	67	9

150 strains were isolated and 76 isolates were identified. Most isolates were Deuteromycetes (moulds). Two basidiomycetes species *Schizophyllum commune* and *Cyathus stercoreus* were found. Figure 1 shows examples of mould appearance on malt agar.

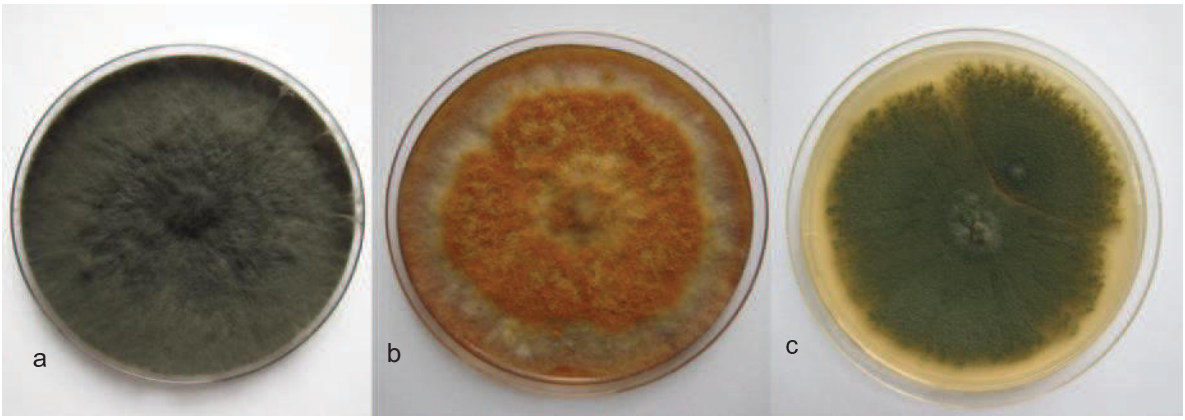

Figure 1. Moulds isolated from bamboo. a: *Botryosphaeria subglobosa*; b: *Epicoccum nigrum*; c: *Penicillium commune*

Table 2. Examples for comparing BLAST identification results based on rDNA-ITS, *ß*-Tubulin and Calmodulin sequences

Fungal strain coding	ITS		*ß*-Tubulin		Calmodulin	
	Species	Max. Identity (%)	Species	Max. Identity (%)	Species	Max. Identity (%)
D54	*Aspergillus nomius*	100	*A. nomius*	99	*A. nomius*	99
			A. zhaoqingensis	99	*A. zhaoqingensis*	99
D23-1	*Penicillium biourgeianum*	100	*P. biourgeianum*	100	*P. biourgeianum*	98
D19-2	*P. brevicompactum*	100	*P. brevicompactum*	98	*P. brevicompactum*	99
D36	*Penicillium* sp.	100	*P. phialosporum*	90	*P. variabile*	82
D80-2	*P. commune*	100	*P. commune*		*P. commune*	99
D46	*P. citrinum*	100	*P. citrinum*	100	*P. citrinum*	100
D9-1	*P. expansum*	100	*P. expansum*	99	*P. expansum*	97
D57	*P. verruculosum*	100	*Penicillium* sp.	87	*P. aculeatum*	87
	P. aculeatum	98			*P. pinophilum*	85
D79-1	*P. expansum*	100	*P. expansum*	100	*P. expansum*	97
D65-1	*P. polonicum*	100	*P. polonicum*	100	*P. commune*	92
					P. thymicola	92
					P. echinulatum	91

Some mould genera like *Aspergillus* and *Penicillium* contain very closely related species of which some have also various synonyms. Doubtful results may be obtained when only the ITS sequence is used for identification. Furthermore, the international DNA data bases are human-made and contain mistakes, like miss-identifications. Therefore, also *ß*-Tubulin and Calmodulin sequences were obtained for those fungi and used for BLAST identification (Table 2).

Whereas some moulds like strains D23-1 and D19-2 revealed identical names with all three DNA sequences other fungi like D54 showed quite different naming. However, it has to be considered that some isolates are obviously not represented in the data bases by all three sequences like *Penicillium*

polonicum D65-1 and Calmodulin. We assume that most of the isolated and identified fungi are accidental infections under the respective use conditions of the culms.

Blue-stain Discolouration of Bamboo Samples

All bamboo samples inoculated with the blue-stain fungi *Alternaria tenuissima*, *Alternaria alternata*, *Aureobasidium pullulans*, *Botryosphaeria subglobosa*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Hormonema dematioides*, *Pestalotiopsis microspora* and *Phoma macrostoma* discoloured to grey-black-blue within four weeks of incubation.

The experimental set-up is demonstrated in Figure 2. Figure 3 shows examples of the light microscopic investigation of the attacked samples.

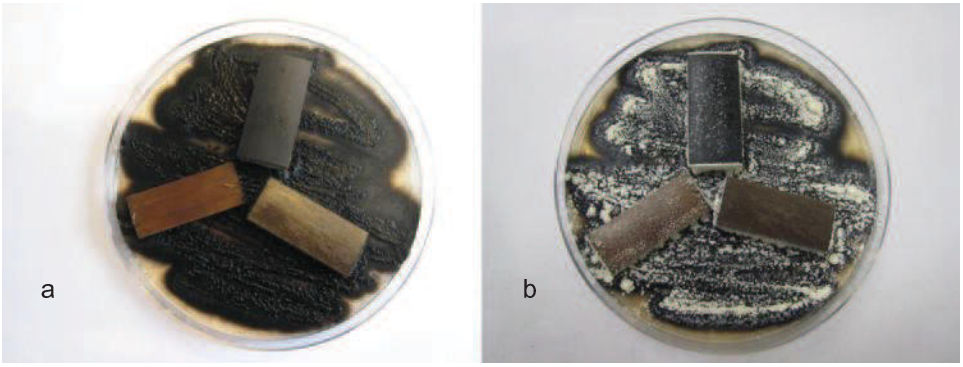

Figure 2. Cultures of the blue-stain fungus *Aureobasidium pullulans* with bamboo samples after 1 month (a) and 5 month (b) of incubation.

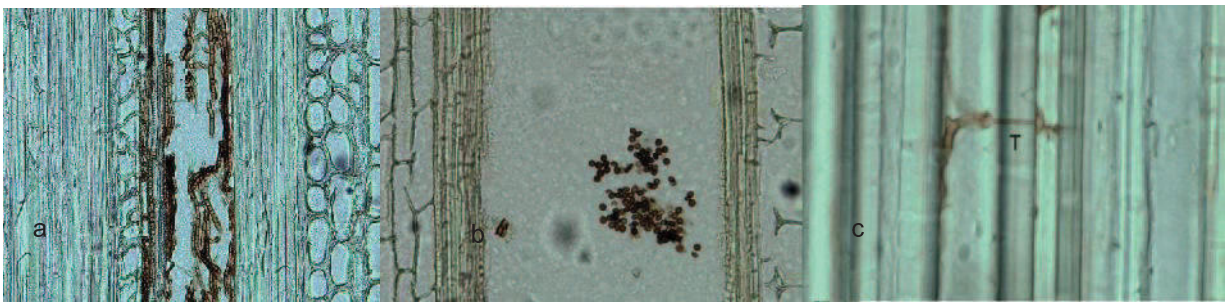

Figure 3. Colonization of bamboo by blue-stain fungi. a: Thick, brown hyphae of *Cladosporium cladosporioides* in a vessel of *Phyllostachys pubescens* (100x); b: brown chlamydospores of *Botryosphaeria subglobosa* in *Gigantochloa atroviolacea* (100x); c: transpressorium (T) of *Alternaria alternata* in *Bambusa maculata* (600x)

The tissue was colonized by the typical thick and brown hyphae and chlamydospores of blue stain fungi. In some cases, a transpressorium, a thin penetration hypha (Fig. 3 right), was observed. The transpressorium is the only fungal `organ´ by which blue stain fungi damage lignified cell walls (Liese and Schmid 1966).

Degradation of Bamboo by Fungi

Figure 4 shows the experimental set-up of decay tests performed in preserving jars.

Table 3 summarizes the % mass loss results after incubation.

Figure 4. a: Preserving jars with bamboo samples; b: samples on mycelium of *Trametes versicolor* after 1 week of incubation

Table 3. Decay of bamboo species (min., average and max. % mass loss of 3 replicates after 1 year of incubation in preserving jars)

Fungus	Rot type	*Bambusa maculata*	*Gigantochloa atroviolacea*	*Phyllostachys pubescens*
Pleurotus ostreatus	White	22.9.....35.7 28.2	8.5.....13.6 10.6	19.7.....22.3 21.0
Schizophyllum commune 87	White	2.7.....2.9 2.8	6.7.....6.8 6.7	4.7....5.6 5.2
S. commune 98	White	1.6.....2.0 1.8	4.5.....7.3 5.6	3.6.....5.1 4.4
Trametes versicolor	White	60.2.....64.0 62.5	45.7.....55.4 51.6	44.5.....54.0 47.8
Coniophora puteana	Brown	2.8.....4.2 3.6	5.2.....5.9 5.6	4.6.....4.8 4.7
Gloeophyllum trabeum	Brown	1.8.....2.0 1.9	4.8.....6.7 5.7	4.3.....7.2 5.3
Chaetomium globosum	Soft	31.2.....32.7 31.8	7.6.....12.7 9.4	36.9.....39.7 38.0
Paecilomyces variotii	Soft	0.8.....1.4 1.2	3.3.....4.8 3.6	3.4.....4.2 3.9

Although the white rot species *Schizophyllum commune* is a common colonizer of bamboos (Liese 1985; Mohanan 1997; Liese and Kumar 2003), it caused only a minor level of decay (also reported by Suprati (2010) and Kim et al. (2011)). Of the other white-rotters, *Pleurotus ostreatus* showed medium degradation and *Trametes versicolor* caused the greatest mass loss of 62.5 %. The brown-rot fungi *Coniophora puteana* and G*loeophyllum trabeum* were relatively inactive, the latter contrasts to Lee et al. (2006) and Ma et al. (2010). Of the soft-rot fungi (cf. Leithoff and Peek 2001), *Chaetomium globosum* produced severe degradation (38%).

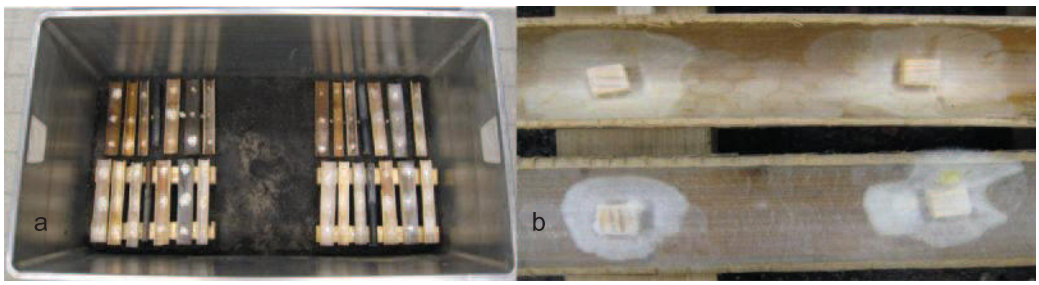

Figure 5. a: Fungus cellar-test with a metal tub and soil; b: samples with *Schizophyllum commune* after 1 week of incubation.

Table 4 summarizes the additional degradation results with the less active fungi *S. commune* and *C. puteana* obtained with the Fungus cellar test.

Table 4. Decay (% mass loss) and final moisture (% u) of bamboo samples in Fungus cellar test

Bamboo species	Tub	Soil contact	*Coniophora puteana* 1		*Schizophyllum commune* 87	
			Mass loss	Moisture	Mass loss	Moisture
Arundinaria	1	+	15.5	187	15.3	148
amabilis	1	-	38.6	41	10.8	31
	2	-	38.7	45	8.7	68
	2	-	39.0	56	7.1	40
Bambusa	1	+	9.9	159	11.0	174
maculata	1	-	20.4	39	5.1	28
	2	-	38.3	48	5.0	33
	2	-	34.3	52	3.6	37
Dendrocalamus	1	+	5.4	104	5.1	90
asper	1	-	29.3	42	4.3	28
	2	-	26.9	45	3.8	32
	2	-	28.5	50	4.3	31
Gigantochloa	1	+	6.1	95	6.1	95
atroviolacea	1	-	18.7	34	4.7	25
	2	-	41.6	58	3.0	38
	2	-	42.9	57	5.0	43
Phyllostachys	1	+	9.7	112	16.4	126
nigra	1	-	32.6	49	9.1	32
	2	-	39.7	65	6.6	40
	2	-	40.7	56	6.4	52
Phyllostachys	1	+	35.3	103	19.7	182
nigra `Boryana´	1	-	38.8	54	6.7	26
	2	-	37.9	60	5.7	40
	2	-	38.8	46	5.1	35
Phyllostachys	1	+	6.3	61	6.3	63
pubescens	1	-	5.4	32	5.4	24
	2	-	38.3	42	5.7	31
	2	-	31.2	43	6.3	36

In contrast to results with small samples in the jar test, bamboo samples in the Fungus cellar were considerably more degraded by *C. puteana* (max. 43%) and *S. commune* (max 20%). Obviously, the moisture conditions in the Fungus cellar test influenced the activity of both fungi, whereby the white-rot fungus *S. commune* differed considerably from the brown-rot species *C. puteana*. *Schizophyllum commune* decayed samples with soil contact and thus with high water content (90-182 % u) more than samples without soil contact. In contrast, *C. puteana* produced a higher mass loss in samples located on wood supports and with lower water content (34-65 % u). Not shown experiments with samples incubated on vermiculite substrate with different water content also demonstrated the influence of moisture on decay.

The tested bamboos differed in susceptibility to fungi (Tables 3 and 4; also Suprati 2010). Furthermore, samples from young culms were more susceptible to decay than older ones (Hamid et al. 2003; Schmidt et al. 2011). Samples from culm top were more decayed than those from the bottom

(Suprati 2010; Schmidt et al. 2011). Sealing the crosscut ends forced the hyphae to penetrate through the epidermis and not through the vessels at the crosscut plane which reduced the rate of decay (Schmidt et al. 2011; also Kleist et al. 2002).

Micromorphological Studies of Degradation

Figure 6 shows examples for the micromorphological degrading patterns of bamboo cell walls. The tissue after brown-rot attack remained nearly intact (Figure 6a). Degradation of lignified cell walls by white-rot fungi (Figure 6b) of the erosion type begins in the lumen (Liese 1970). Excretion of enzymes caused wholes and openings in the cell wall. A conspicuous feature of bamboo is the frequent occurrence of hyphae in the interstitial region. The soft-rot decay (Figure 6c) resembles a late stage soft-rot in wood. Only the highly lignified middle lamella/primary walls and the tertiary wall remained; the secondary wall with the soft-rot hyphae inside was totally destructed to residues. The findings extend previous work on the few micromorphological degradation studies of bamboo by wood-destroying fungi, which were only observed in the brown-rot species *Gloeophyllum trabeum* (Lee et al. 2006) and the white-rot mushroom *Lentinula edodes* (Ma et al. 2010).

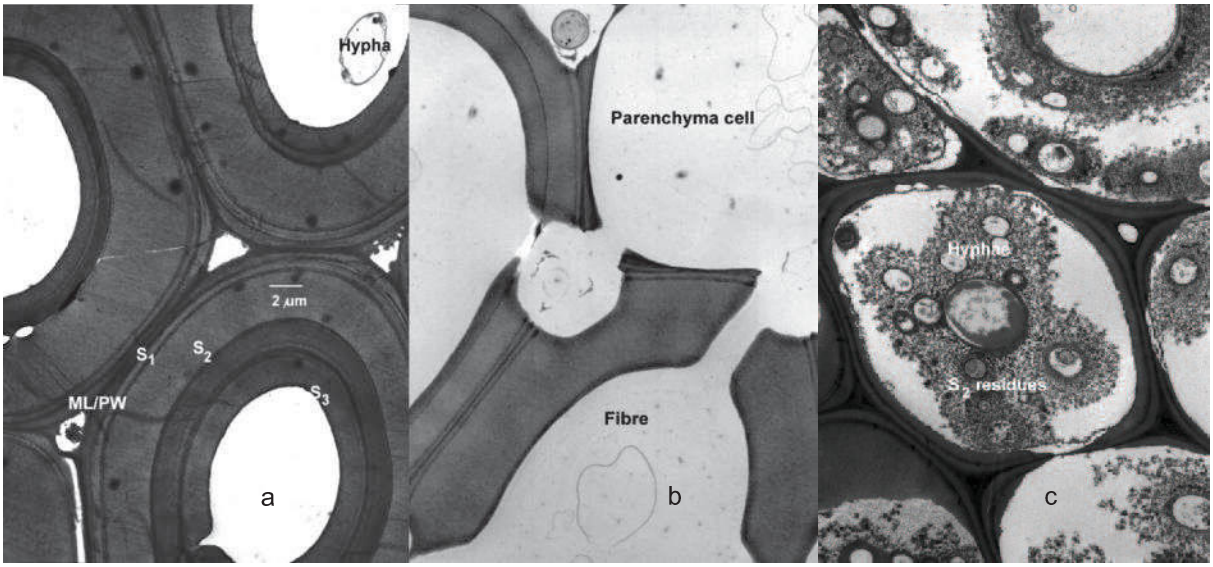

Figure 6. a: Early brown-rot symptoms in *Gigantochloa atroviolacea* by *Coniophora puteana*; b: medium white-rot decay in *Bambusa maculata* by *Trametes versicolor*; c: severe soft-rot degradation in *B. maculata* by *Chaetomium globosum*

In summary, the results show that all bamboo species investigated can be colonized by the various groups of fungi, moulds, staining and rot fungi. Considerable degradation occurs by white-, brown- and soft-rot fungi.

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References

Abdurachim Martawidjaja, R.A. 1964. Bamboo preservation in Indonesia. Rimba Indonesia, 9, 66-76.

Banerjee, S.; Mukhopadhya, S.A. 1962. A study on *Merulius similis* B. & FR. and the associated bamboo rot. Oestereichische Botanische Zeitschrift, 109, 197-212.

EN 113. 1996. Determination of the toxic values of wood preservatives against wood destroying Basidiomycetes cultured on agar medium. Beuth, Berlin, Germany. 15 pp.

Hamid, N.H.; Abd. Latif, M.; Sulaiman, O. 2003. Decay resistance of bamboo (*Gigantochloa scortechinii*) compared to 24 Malaysian hardwood. XII. World Forestry Congress 2003, Québec, Canada, www.fao.org.docrep/article/wfc/XII/0039-b4.htm

Kim, J.S.; Lee, K.H.; Cho, C.H.; Koch, G.; Kim, Y.S. 2008. Micromorphological characteristics and lignin distribution in bamboo (*Phyllostachys pubescens*) degraded by the white rot fungus *Lentinus edodes*. Holzforschung, 62(4), 481-487.

Kim, J.J.; Lee, S.S.; Ra, J.B.; Lee, H.; Huh, N.; Kim, G.H. 2011. Fungi associated with bamboo and their decay capabilities. Holzforschung , 65(2), 271-275.

Kleist, G.; Morris, I.; Murphy, R. 2002. Invasion and colonization of bamboo culm material by stain and decay fungi. IRG Working Paper No. 02-10453. International Research Group on Wood Preservation, Stockholm, Sweden. 10 pp.

Lee, K.H.; Cho, C.H.; Kim, Y.S. 2006. Micromorphology of bamboo fibers degraded by brown-rot fungus *Gloeophyllum trabeum*. IRG Working Paper No. 06-10576. International Research Group on Wood Preservation, Stockholm, Sweden. 9 pp.

Leithoff, H.; Peek, R. 2001. Heat treatment of bamboo. IRG Working Paper No. 01-40216. International Research Group on Wood Preservation, Nara, Japan. 11 pp.

Liese, W. 1959. Bamboo preservation and soft-rot. FAO Report to the Government of India 1106, 36 pp.

Liese, W. 1970. Ultrastructural aspects of woody tissue disintegration. Annual Review Phytopathology, 8, 231-258.

Liese, W. 1985. Bamboos - biology, silvics, properties, utilization. Schriftenreihe Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany, 180, 132 pp.

Liese, W.; Kumar, S. 2003. Bamboo preservation compendium. INBAR, Beijing, China, Technical Report 22. 231 pp.

Liese, W.; Schmidt, R. 1966. Untersuchungen zum Zellwandabbau von Nadelholz durch *Trametes pini*. Holz als Roh- und Werkstoff, 24, 454-460.

Ma, X.; Jiang, M.; Qin, D. 2010. The invasion channels of damage fungi in bamboo lumber. IRG Working Paper No. 10-10712. International Research Group on Wood Preservation, Biarritz, France. 7 pp.

Mohanan, C. 1997. Diseases of bamboos in Asia. International Development Research Centre, New Delhi, India. 228 pp.

Murphy, R.J.; Alvin, K.L.; Tan, Y.F. 1991. Development of soft rot decay in the bamboo *Sinobambusa tootsik*. IAWA Bulletin n. s. 12, 85-94.

Purushotham, A. 1963. Utilization of bamboo. Journal Timber Development Preservation Association, 92, 2-19.

Razak, W.; Hashim, W.S; Murphy, R.J. 2002. SEM observation on the decay of bamboo *Gigantochloa scortechinii* exposed in tropical soil. Journal Tropical Forest Products, 8, 168-178.

Savory, J.G. 1954. Damage of wood caused by micro-organisms. Journal Applied Microbiology, 17, 213-218.

Schmidt, O.; Grimm, K.; Moreth, U. 2002. Molecular identity of species and isolates of the *Coniophora cellar* fungi. Holzforschung, 56(6), 563-571.

Schmidt, O.; Wei, D.S.; Liese, W. 2011. Fungal degradation of bamboo samples. Holzforschung, 65(6), 883-888.

Spurr, A.R. 1969. A low viscosity embedding medium for electron microscopy. Journal Ultrastructural Research, 26, 31-43.

Suprapti, S. 2010. Decay resistance of five Indonesian bamboo species against fungi. Journal Tropical Forrest Science, 22, 287-294.

Thang, T.K.H.; Schmidt, O.; Liese, W. 2009. Environment-friendly short-term protection of bamboo against moulding. Journal Timber Development Association India, 55, 8-17.

Wang, S.F.; Hsieh, R.C. 1968. Durability records of treated and untreated bamboo. Cooperation Bulletin Taiwan Forest Research Institute, Taiwan 15, 26 pp.

White, T.J.; Bruns, T.; Lee, S.; Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A.; Gelfand, D.H.; Sninisky, J.J.; White, T.J. (eds.). PCR protocols. Academic Press, San Diego, 315.322.

Zang, X.; Yu, H.; Huang, H.; Liu, Y. 2007. Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culm. International Biodeterioration Biodegradation, 60, 159-164.

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ORIGINALS ORIGINALARBEITEN

Durability test of bamboo against fungi according to EN standards

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Abstract The durability of five bamboo species from various origin against brown-, white- and soft-rot fungi was investigated in Kolle flasks in accordance with the European standards EN 350-1, EN 350-2 and EN 113. Considerable variability exists in the durability of the bamboo species. *Guadua angustifolia* was rather resistant to *Trametes versicolor* and *Dendrocalamus asper* against *Chaetomium globosum*. Among the brown-rot fungi, the four strains of *Coniophora puteana* and two strains of *Gloeophyllum trabeum* produced low mass loss (maximum 2.9 %). Of the white-rot fungi, *T. versicolor* yielded the highest decay (max. 15.3 %), whereas *Schizophyllum commune* was rather inactive (max. 3.2 %). Of the soft-rot fungi, *Ch. globosum* showed medium degradation (max. 9.6 %) and *Paecilomyces variotii* low decay (max. 3.1 %).

Dauerhaftigkeitsprüfung von Bambus gegenüber Pilzen gemäß EN-Standards

Zusammenfassung Fünf Bambus-Arten verschiedener Herkunft wurden in Kolleschalen auf ihre Anfälligkeit für Braun-, Weiß- und Moderfäulepilze gemäß EN 350-1, EN 350-2 und EN 113 untersucht. Die Bambus-Arten unterschieden sich in ihrer Pilzanfälligkeit. *Guadua angustifolia* war besonders widerstandsfähig gegen *Trametes versicolor* und *Dendrocalamus asper* gegen *Chaetomium globosum*. Bei den Braunfäule-Erregern ergaben die vier Stämme von *Coniophora puteana* und beide Isolate von *Gloeophyllum trabeum* nur geringen Masseverlust (maximal 2,9 %). Von den beiden Weißfäulepilzen zeigte *T. versicolor* den stärksten Abbau (max. 15,3 %), während *Schizophyllum commune* kaum aktiv war (max. 3,2 %). Bei den Moderfäulepilzen ergab *Ch. globosum* mittleren (max. 9,6 %) und *Paecilomyces variotii* geringen Abbau (max. 3,1 %).

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1 Introduction

Bamboo is a fast growing woody grass of increasing interest for the sustainable production of materials with many applications for buildings and industrial utilization. However, bamboo has generally a low natural durability and is easily attacked by fungi during storage, transport and final use. For applications it is important to know its susceptibility.

Like other lignocellulose materials, bamboos are subject to biodegradation by fungi under particular conditions which affects their quality (Hamid et al. 2003). The resistance of bamboo against decay fungi serves as an important parameter in bamboo uses. Several factors may influence bamboo natural durability, for example site, growth rate, age, portion of bamboo, extractives content and the microenvironment. The results obtained in this work, together with other data reported in literature on the decay of bamboo (Liese 1959, 1985; Banerjee and Mukhopadhyay 1962; Purushotham 1963; Abdurachim 1964; Wang and Hsieh 1968; Murphy et al. 1991; Razak et al. 2002, 2006; Hamid et al. 2003; Zhang et al. 2007; Kim et al. 2008, 2011; Suprapti 2010; Ma et al. 2010; Schmidt et al. 2011; Wei et al. 2012) may contribute to the characterization and appreciation of bamboo species. Such characterization is important not only for its correct utilization but also for the market promotion.

When comparing data, another difficulty is due to the different tests performed, such as field and laboratory tests. Also within the same test, the different standards (Asia,

Table 1 Fungi investigated
Tab. 1 Untersuchte Pilze

Rot type	Species	Laboratory isolate coding	Other codings/ use for EN	Origin/isolation by
Brown	*Coniophora puteana*	1	Ebw. 15/EN 113	Building, Berlin, Germany/J. Liese 1930
	Coniophora puteana	159		Wood, Soerfosa, Sweden/T. Nilsson 1995
	Coniophora puteana	167		Building, Hamburg, Germany/O. Schmidt 1997
	Coniophora puteana	247		Building, Karlsruhe, Germany/K. Grimm 1990
	Gloeophyllum trabeum	183	Ebw. 109/EN 113	Building, Eberswalde, Germany/J. Liese
	Gloeophyllum trabeum	259		Cellar stairs, Lörrach, Germany/K. Grimm 1999
White	*Trametes versicolor*	63	CTB 863/EN 113	France
	Schizophyllum commune	87		*Dendrocalamus brandisii*, Costa Rica/D. Wei 2009
Soft	*Chaetomium globosum*	10	ATCC 44753	Wood, Munich, Germany/1963
	Paecilomyces variotii	13	ATCC 44741	Sugar cane bagasse, Trinidad/K. Walter 1977

European and American) may differ by sample size, duration of tests and fungal strains. Therefore, it is important to test the bamboo species using the same standards. The aim of the study is to test bamboo durability using European standards as used for wood species.

2 Materials and methods

2.1 Investigated fungi

The ten strains of white-, brown- and soft-rot fungi used derive from the laboratory strain collection and are listed in Table 1.

2.2 Bamboo and wood samples

The five bamboo species tested are presented in Table 2. Culm sections were obtained from CONBAM, Geilenkirchen, Germany and the Bamboo Centre, Baden–Baden, Germany. Sections were proved with the curcuma-test (Peylo 2001) to ensure that no boron was present which is commonly used against moulding during storage and transport. Samples were prepared by cutting to dimensions of 5 (length) × 2.5 (width) × 0.5–3.5 cm (wall thickness). Wood samples from *Fagus sylvatica* and *Pinus sylvestris* were used as controls according to EN 113. All samples were put into a climate room at 20 ± 2 °C and 65 ± 5 % relative humidity (RH) for 4 weeks, weighed, wrapped in plastic (Sengewald Flexopeel, Germany) and send to BBF Sterilisationsservice GmbH, Kernen, Germany for gamma radiation.

Table 2 Bamboo species tested
Tab. 2 Untersuchte Bambus-Arten

Bamboo species	Local name	Origin
Bambusa maculata	Tutul	Indonesia (Java)
Dendrocalamus asper	Petung	Indonesia (Java)
Gigantochloa atroviolacea	Wulung	Indonesia (Java)
Guadua angustifolia	Guadua	Columbia
Phyllostachys pubescens	Moso	China
Phyllostachys pubescens		Germany

2.3 Durability test

The durability experiment was performed with fungal pure cultures in Kolle flasks with white-, brown-, and soft-rot fungi according to the European standards EN 350-1 (1996), EN 350-2 (1997) and EN 113 (1996). The culture medium consisted of 2 % malt extract (Merck) and 1.5 % agar (Bacto) in distilled water. Flasks containing 48 ml medium were plugged with cotton, sterilized in the autoclave at 121 °C for 30 min, inoculated with a mycelial plug of the test fungus and incubated until mycelial growth covered the surface of the medium. Each two gamma ray sterilized samples were put aseptically in one flask and each three flasks were used for one fungal strain, with altogether 480 Kolle flasks. After 16 weeks of incubation at 20 ± 2 °C and 65 ± 5 % RH for the brown-rot and white-rot fungi and 28 ± 2 °C and 65 ± 5 % RH for the soft-rot fungi, the mycelium was removed from the sample surface, the samples were weighed for final wet weight determination and oven dried at 103 °C. Percentage of mass loss was calculated by weight comparison before and after incubation. Statistical differences were analyzed using variance analysis (ANOVA, SPSS software 2002).

2.4 Assessment of fungal growth and classification of bamboo durability

The development of fungal growth on the specimens was evaluated by the density and the percentage of the sample surface covered with mycelium (Table 3). Based on the

Table 3 Mycelium growth and durability class after 16 weeks of incubation
Tab. 3 Mycelwachstum und Dauerhaftigkeitsklasse nach 16 Wochen Kultivierung

	Bambusa maculata			*Dendrocalamus asper*			*Gigantochloa atroviolacea*			*Guadua angustifolia*			*Phyllostachys pubescens* China			*Phyllostachys pubescens* Germany		
	HD	HC	DC	HD	HC	DC	HD	HC	DC	HD	HC	DC	HD	HC	DC	HD	HC	DC
Coniophora puteana 1	2	4	II	1	3	II	2	4	II	1	0	II	2	4	II	3	4	II
Coniophora puteana 159	2	4	II	2	4	II	2	4	II	2	2	II	2	4	II	2	4	II
Coniophora puteana 167	2	4	II	1	4	II	2	4	II	1	1	II	2	4	II	2	4	II
Coniophora puteana 247	2	4	II	2	4	II	2	4	II	0	0	II	2	4	II	2	4	II
Gloeophyllum trabeum 183	0	0	II	0	0	II	1	4	II	0	0	II	1	4	II	1	4	II
Gloeophyllum trabeum 259	1	2	II	1	4	II	1	4	II	0	0	II	1	3	II	1	4	II
Trametes versicolor	1	4	III	2	4	III	2	4	IV	1	2	II	3	4	IV	2	4	IV
Schizophyllum commune	2	4	II	1	4	II	2	4	II	0	0	II	2	4	II	2	4	II
Chaetomium globosum	2	4	III	2	4	II	2	4	III	1	2	III	2	4	III	2	4	II
Paecilomyces variotii	2	4	II	2	4	II	2	4	II	0	0	II	2	4	II	2	4	II

HD Hyphal density: 0, no growth; 1, sparse mycelium; 2, normal mycelium; 3, thick mycelium

HC Hyphal coverage: 0, no coverage; 1, 1–33 % coverage; 2, 34–66 % coverage; 3, 67–99 % coverage; 4, total coverage

DC Durability class: II, durable (mass loss <5 %); III, moderately durable (5–10 %); IV, little durable (10–30 %)

average mass loss, bamboo durability against fungi was correlated to durability classes (Table 3) considering EN 350-1 (1996), Abdurachim (1975) and Djarwanto and Suprapti (2004).

3 Results and discussion

3.1 Growth of fungi and final moisture content of bamboo samples

The development of hyphal growth was measured by scoring the density of hyphal mats and the percentage coverage of the sample surface. After initial differences between the fungi within the first 2 weeks, most bamboo species were covered by mycelium after 16 weeks between two-thirds of the surface and total coverage (Table 3). An exception with low surface growth occurred on *Guadua angustifolia*. In general, coverage correlated with hyphal density. The density of mycelium is a specific feature for many fungi (Stalpers 1978). However, it must not relate to fungal activity (Schmidt 2006). Among the ten strains, *Gloeophyllum trabeum* 183 did not show any hyphae on the surface of samples from *Bambusa maculata, Dendrocalamus asper* and *Guadua angustifolia*. However, this strain produced some degradation (Fig. 1), obviously not by surface growth but by substrate mycelium. The final moisture content of the samples ranged from 39 to 101 %, which is generally a suitable range for fungal degradation. Former experiments showed fungal bamboo degradation in the moisture range from 24 to 187 % (Schmidt et al. 2011).

3.2 Durability of bamboo against brown-rot fungi

All five bamboo species were rather resistant to degradation by the various strains of the brown-rot fungi *Coniophora puteana* and *Gloeophyllum trabeum* (Fig. 1) without significant variation among the strains. Strain variation with regard to wood mass loss was shown for both species (Schmidt et al. 2002a, 2002b). Both fungi comprised the test strains to be used in EN 113 (1996) for wood samples. In this standard, *C. puteana* strain Ebw. 15 produces about 20 % mass loss in wood and wood products. Among the four *C. puteana* strains, the maximum mass loss of bamboo was 2.9 %. The two *G. trabeum* strains also revealed no significant difference in bamboo degradation, regardless of the species. Mass losses of the wood controls for the four *C. puteana* strains and two *G. trabeum* isolates ranged from 19.7 to 62.7 % of decay. This shows suitable incubation conditions for bamboo. The low activity against bamboo correlates with previous work by the authors (Schmidt et al. 2011; Wei et al. 2012) reporting maximum 5.7 % mass loss by both fungi for the same bamboo species of the same origin after 1 year of incubation in preserving jars. However, samples of *Melocanna bambusoides* after 6 month incubation in Kolle flasks had shown up to 13.7 % decay by *C. puteana* Ebw. 15 (Schmidt et al. 2011). To prove these earlier results, the degradation test according to EN 350 and EN 113 considered sample sterilization by gamma radiation. The highest mass loss of 3 % by *G. trabeum* is lower than the one reported by Schmidt et al. (2011) with maximum 5.7 % of decay after 1 year in preserving jars. However, both results contrast with Lee et al. (2006) who used potato dextrose agar in petri dishes and found 25 %

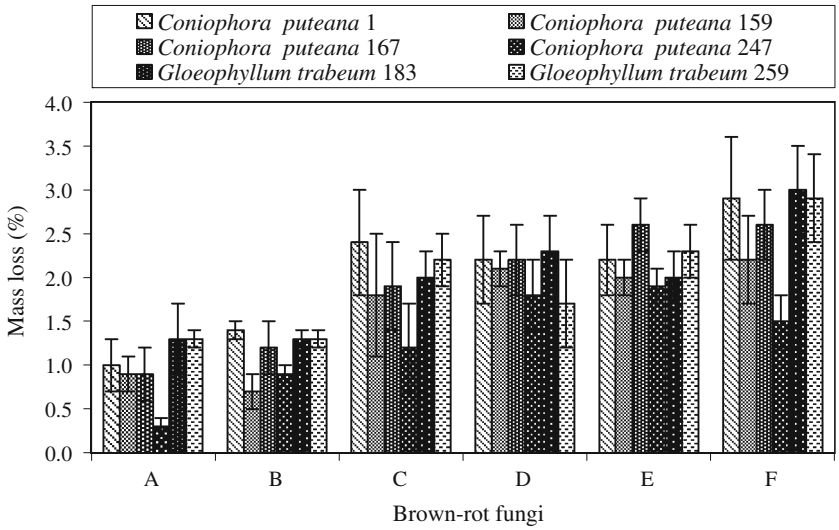

Fig. 1 Mass loss caused by brown-rot fungi in 5 bamboo species after 16 weeks of incubation. *Error bars* represent mean ± standard deviation. **A** *Bambusa maculata*; **B** *Dendrocalamus asper*; **C** *Gigantochloa atroviolacea*; **D** *Guadua angustifolia*; **E** *Phyllostachys pubescens* China; **F** *Phyllostachys pubescens* Germany

Abb. 1 Masseverluste durch Braunfäulepilze bei 5 Bambus-Arten nach 16 Wochen Inkubation. Fehlerbalken zeigen ± Standardabweichung. **A** *Bambusa maculata*; **B** *Dendrocalamus asper*; **C** *Gigantochloa atroviolacea*; **D** *Guadua angustifolia*; **E** *Phyllostachys pubescens* China; **F** *Phyllostachys pubescens* Deutschland

degradation by *G. trabeum* in *Phyllostachys pubescens*. The reason for this discrepancy may be the use of different isolates and cultivation methods.

3.3 Durability of bamboo against white-rot fungi

Among the two white-rot fungi, *Trametes versicolor* showed the greatest mass loss with 15.3 % for *P. pubescens* from China, followed by *P. pubescens* from Germany (12.3 %) and *Gigantochloa atroviolacea* (10.4 %) (Fig. 2). *Guadua angustifolia* was very resistant (2.3 %). Degradation of the *Fagus sylvatica* control was 26.7 %. *Schizophyllum commune* behaved rather inactive with maximum mass loss of only 3.2 %. Schmidt et al. (2011) obtained with *S. commune* a maximum of up to 9.1 % degradation in 6 months in Kolle flasks and a maximum of 5.6 % mass loss during 1 year in preserving jars. Abdurachim (1964) reported 15 % mass loss by *S. commune*. Low decay rates by *S. commune* were found by Suprapti (2010) and Kim et al. (2011). The beech wood control in this experiment showed also negligible decay (0.8 %), which corresponds to previous results (Schmidt and Liese 1978). However, this species is common on bamboo during storage and use (Liese 1985; Mohanan 1997; Liese and Kumar 2003). Kleist et al. (2002) showed that *S. commune* was the most successful coloniser among some fungi as it could penetrate bamboo via outer and inner culm walls, through cross section planes as well as through nodal ridges and longitudinally through nodes.

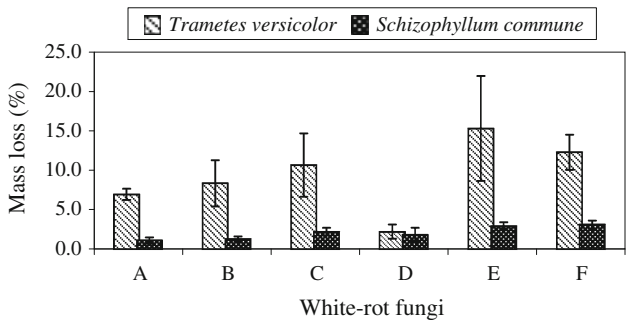

Fig. 2 Mass loss caused by white-rot fungi in 5 bamboo species after 16 weeks of incubation. *Error bars* represent mean ± standard deviation. **A** *Bambusa maculata*; **B** *Dendrocalamus asper*; **C** *Gigantochloa atroviolacea*; **D** *Guadua angustifolia*; **E** *Phyllostachys pubescens* China; **F** *Phyllostachys pubescens* Germany

Abb. 2 Masseverluste durch Weißfäulepilze bei 5 Bambus-Arten nach 16 Wochen Inkubation. Fehlerbalken zeigen ± Standardabweichung. **A** *Bambusa maculata*; **B** *Dendrocalamus asper*; **C** *Gigantochloa atroviolacea*; **D** *Guadua angustifolia*; **E** *Phyllostachys pubescens* China; **F** *Phyllostachys pubescens* Deutschland

3.4 Durability of bamboo against soft-rot fungi

The two soft-rot fungi, *Chaetomium globosum* and *Paecilomyces variotii* revealed significant differences with regard to bamboo decay, with higher mass loss by *Ch. globosum* than by *P. variotii* (Fig. 3). Highest mass loss was caused by *Ch. globosum* to *P. pubescens* from China (9.6 %), followed by *Guadua angustifolia* (8.1 %) and *Bambusa maculata* (7.5 %). Schmidt et al. (2011) reported up to 52.7 % decay by *Ch. globosum* after 6 months in

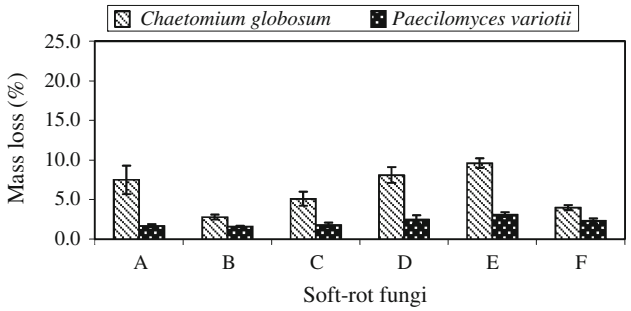

Fig. 3 Mass loss caused by soft-rot fungi in 5 bamboo species after 16 weeks of incubation. *Error bars* represent mean ± standard deviation. **A** Bambusa *maculata*; **B** *Dendrocalamus asper*; **C** *Gigantochloa atroviolacea*; **D** *Guadua angustifolia*; **E** *Phyllostachys pubescens* China; **F** *Phyllostachys pubescens* Germany

Abb. 3 Masseverluste durch Moderfäulepilze bei 5 Bambus-Arten nach 16 Wochen Inkubation. Fehlerbalken zeigen ± Standardabweichung. **A** *Bambusa maculata*; **B** *Dendrocalamus asper*; **C** *Gigantochloa atroviolacea*; **D** *Guadua angustifolia*; **E** *Phyllostachys pubescens* China; **F** *Phyllostachys pubescens* Deutschland

Kolle flasks and maximum 38 % after 1 year in preserving jars. Suprapti (2010) measured 8.0 % mass loss. Kim et al. (2011) obtained up to 17.9 % decay for other soft-rot species. Maximum mass loss by *P. variotii* was only 3.1 %. The same strain of the species had shown maximum 3.9 % degradation in preserving jars, but another strain up to 19.7 % decay in Kolle flasks (Schmidt et al. 2011). The wood controls in this experiment revealed only rather low mass losses around 1 % which explains that soft-rot degradation of wood samples is tested by the specific standard EN 807 (2001).

3.5 Classification of bamboo durability

The bamboos were grouped into 5 durability classes as it is done for wood species. Table 3 shows the durability classification of all bamboo/fungus combinations derived from Figs. 1, 2, 3. The bamboo species investigated belong to 3 different classes. There were considerable differences. The resistances of the five bamboo species to the brown-rot fungi *C. puteana* and *G. trabeum*, the white-rot fungus *S. commune* and the soft-rot species *P. variotii* would group them into class II (durable). The soft-rot fungus *Ch. globosum* attacked the bamboos according to classes II and III (moderately durable). Considering white-rot decay by *T. versicolor*, the five bamboo species varied between classes II and IV (little durable).

4 Conclusion

Bamboo species differ in their susceptibility to fungal decay. With regard to the different groups of rot fungi, bamboo seems to be rather resistant against brown-rot fungi, whereas some soft-rot and white-rot fungi produce considerable deterioration. Results correspond to findings by other authors in laboratory studies and to observations in the practice, where bamboo is often colonized and deteriorated by fungi.

Acknowledgments We thank Wolfgang Eberts (Bamboo Centre Germany) and Christoph Tönges (CONBAM, Germany) for providing bamboo sections and Marie-Therese Lenz for technical help.

References

Abdurachim MRA (1964) Bamboo preservation in Indonesia. Rimba Indonesia 9:66–76

Abdurachim MRA (1975) Laboratory testing on the resistance of wood against fungi. Ministry of Agriculture, Jakarta

Banerjee S, Mukhopadhyay S (1962) A study on *Merulius similis* B. & BR. and the associated bamboo-rot. Öster Bot Zeitschr 109:197–212

Djarwanto, Suprapti S (2004) Laboratory testing on the resistance of wood against fungi. In: Herjanto E et al. (Eds) Prosiding Pertemuan dan Presentasi Ilmiah Standardisasi. Jakarta, pp 15–22

EN 113 (1996) Determination of the toxic values of wood preservatives against wood destroying Basidiomycetes cultured on agar medium. Beuth, Berlin

EN 350-1 (1996) Durability of wood and wood-based products. Natural durability of solid wood. Part 1: Guide to the principles of testing and classification of natural durability of wood. Beuth, Berlin

EN 350-2 (1997) Durability of wood and wood-based products. Natural durability of solid wood. Part 2: Guide to natural durability and treatability of selected wood species of importance in Europe. Beuth, Berlin

EN 807 (2001) Wood preservatives—Determination of the effectiveness against soft rotting micro-fungi and other soil inhabiting micro-organisms. Beuth, Berlin

Hamid NH, Abd Latif M, Sulaiman O (2003) Decay resistance of bamboo (*Gigantochloa scortechinii*) compared to 24 Malaysian hardwood. XII. World Forestry Congress 2003, Québec, Canada. www.fao.org/docrep/article/wfc/XII/0039-b4.htm

Kim JS, Lee KH, Cho CH, Koch G, Kim YS (2008) Micromorphological characteristics and lignin distribution in bamboo (*Phyllostachys pubescens*) degraded by the white rot fungus *Lentinus edodes*. Holzforschung 62:481–487

Kim JJ, Lee SS, Ra JB, Lee H, Huh N, Kim GH (2011) Fungi associated with bamboo and their decay capabilities. Holzforschung 65:271–275

Kleist G, Morris I, Murphy R (2002) Invasion and colonization of bamboo culm material by stain and decay fungi. The International Research Group on Wood Preservation, IRG Document No. IRG/WP 02-10453

Lee KH, Cho CH, Kim YS (2006) Micromorphology of bamboo fibers degraded by brown-rot fungus *Gloeophyllum trabeum*. The International Research Group on Wood Preservation, IRG Document No. IRG/WP 06-10576

Liese W (1959) Bamboo preservation and soft-rot. Food and Agriculture Organization Report to the Government of India, FAO Document No. 1106

Liese W (1985) Bamboos—biology, silvics, properties, utilization. Schriftenreihe Gesellschaft für Technische Zusammenarbeit, GTZ Document No. 180

Liese W, Kumar S (2003) Bamboo preservation compendium. International Network for Bamboo and Rattan, INBR Document No. 22

Ma X, Jiang M, Qin D (2010) The invasion channels of damage fungi in bamboo lumber. The International Research Group on Wood Preservation, IRG Document No. IRG/WG 10-10712

Mohanan C (1997) Diseases of bamboos in Asia. International Development Research Centre, New Delhi

Murphy RJ, Alvin KL, Tan YF (1991) Development of soft rot decay in the bamboo *Sinobambusa tootsik*. IAWA Bulletin n s 12:85–94

Peylo A (2001) Schnellanalyse von Holzschutzmitteln. Schützen & Erhalten:25–27

Purushotham A (1963) Utilization of bamboo. J Timber Dry Pres Assoc India 9:2–19

Razak W, Hashim WS, Murphy RJ (2002) SEM observation on the decay of bamboo *Gigantochloa scortechinii* exposed in tropical soil. J Trop For Prod 8:168–178

Razak W, Mahmud S, Tamizi M, Awang AMY (2006) Durability performance of *Gigantochloa scortechinii* through laboratory fungal decay tests. Res J Microbiol 1:198–202

Schmidt O (2006) Wood and tree fungi. Biology, damage, protection, and use. Springer, Berlin

Schmidt O, Liese W (1978) Biological variations within *Schizophyllum commune*. Material Organisms 11:215–230

Schmidt O, Grimm K, Moreth U (2002a) Molecular identity of species and isolates of the *Coniophora* cellar fungi. Holzforschung 56:563–571

Schmidt O, Grimm K, Moreth U (2002b) Molekulare und biologische Charakterisierung von *Gloeophyllum*-Arten in Gebäuden. Z Mykol 68:141–152

Schmidt O, Wei D, Liese W, Wollenberg E (2011) Fungal degradation of bamboo samples. Holzforschung 65:883–888

Stalpers J (1978) Identification of wood-inhabiting Aphyllophorales in pure culture. Studies Mycol 16:1–248

Suprapti S (2010) Decay resistance of five Indonesian bamboo species against fungi. J Trop For Sci 22:287–294

Wang SF, Hsieh RC (1968) Durability records of treated and untreated bamboo. Coop Bull Taiwan For Res Inst 15:26

Wei D, Schmidt O, Liese W (2012) Susceptibility of bamboo to fungi. IXth World Bamboo Congress, pp 235–245

Zhang X, Yu H, Huang H, Liu Y (2007) Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culm. Int Biodeterior Biodegrad 60:159–164

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METHOD TO TEST FUNGAL DEGRADATION OF BAMBOO AND WOOD USING VERMICULITE AS RESERVOIR FOR MOISTURE AND NUTRIENTS

Dongsheng Wei [1,♠], Olaf Schmidt[1], Walter Liese[1]

ABSTRACT

Proposed is a method to investigate degradation of lignocelluloses by pure cultures of basidiomycetes using preserving jars with vermiculite as reservoir for water and nutrients. Bamboo samples of *Gigantochloa atroviolacea* and *Phyllostachys pubescens* and wood samples of *Fagus sylvatica* and *Pinus sylvestris* were inoculated with the brown-rot fungus *Coniophora puteana* and the white-rot fungus *Schizophyllum commune*. The fungi were cultured on vermiculite containing different amounts of tap water or malt extract solution. Mass loss of the bamboos after 32 weeks was low and did not show a remarkable influence of moisture content and nutrient addition. However, considerable degradation of *Pinus sylvestris* sapwood occurred by *C. puteana* whereby moisture and nutrients influenced aggressiveness.

Keywords: Bamboo degradation, vermiculite, moisture content, nutrient addition.

INTRODUCTION

Bamboo is a fast growing woody grass with increasing importance for sustainable production of materials with many applications for structures and industrial utilization. However, bamboo has a low natural durability and is attacked by fungi during storage, transport, processing and final use (Liese and Kumar 2003). There are several reports on its degradation by fungi (Liese 1959, 1985; Abdurachim 1964, Mohanan 1997, Kim *et al.* 2011, Suprapti 2010, Ma *et al.* 2010, Schmidt *et al.* 2011). The behaviour of bamboo against decay fungi is an important parameter in bamboo establishment and use. Most investigations revealed that bamboo degradation is due to white and soft rot fungi, whereas brown-rot species were less aggressive. Among the investigated fungi, *Coniophora puteana* and *Schizophyllum commune* varied with respect to the test method used: Low maximum mass loss was measured on agar under pure culture condition, whereas considerable degradation occurred in the `fungus cellar test´ where the samples are placed in large metal containers on unsterile garden soil with different moisture accessibility (Schmidt *et al.* 2011). However, *C. puteana* and *S. commune* differed in decay activity: *C. puteana* produced maximum mass loss at low moisture content of 57%, whereas decay by *S. commune* was highest at 182% moisture content (Schmidt *et al.* 2011).

Mixed microbial decay in soils with various water holding capacities was studied by Becker and Kaune (1966). Kaune (1970) and Worrall and Wang (1991) used for wood a vermiculite system with pure cultures of soft-rot fungi and proved its suitability for wood degradation tests. Adaskaveg *et al.* (1991) showed decay of palm wood by basidiomycetes with the vermiculite-block assay. Curling *et al.* (2002) exposed basidiomycetes to wood in a vermiculite-soil system. The American standard soil-block test ASTM D1413 was not used for our study because it is principally similar to our former fungus cellar test (Schmidt *et al.* 2011).

The aim of this study was to test bamboo and wood degradation by pure cultures of basidiomycetes with vermiculite as reservoir of moisture and nutrients.

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MATERIALS AND METHODS

Fungi

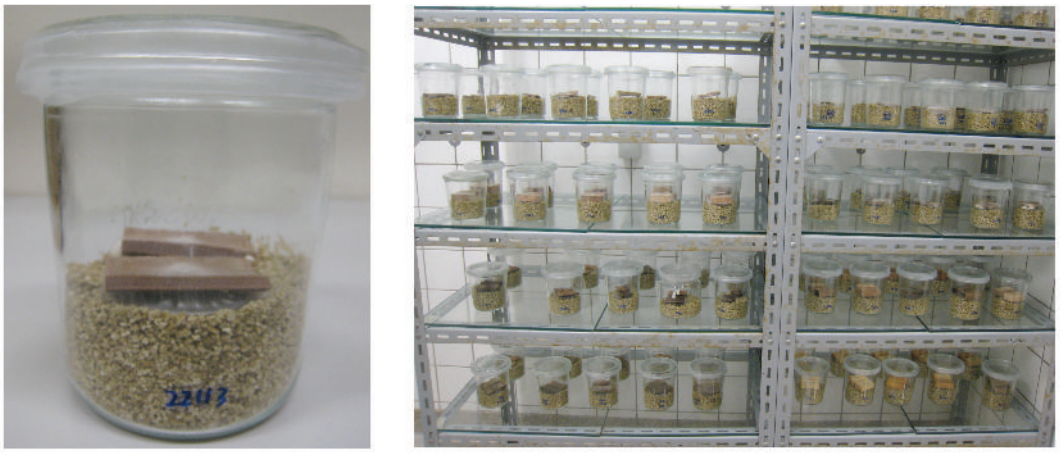
The brown-rot fungus *Coniophora puteana* strain 167 from the laboratory collection and the white-rot fungus *Schizophyllum commune* strain 87 isolated from bamboo (Schmidt *et al.* 2011) were held on agar plates of 2% malt extract (Oxoid) and 1.5% agar (Oxoid).

Bamboo and wood samples

Bamboo species *Gigantochloa atroviolacea* and *Phyllostachys pubescens* were used. Culm sections of *G. atroviolacea* (diameter 5.5-7.0 cm) and *P. pubescens* (diameter 5.5-6.5 cm) were obtained from CONBAM, Geilenkirchen, Germany and the Bamboo Centre, Baden-Baden, Germany, respectively. Culms were proven with the curcuma-test (Peylo 2001) to ensure that no boron was present which is commonly used against moulding. The samples were cut to 5 cm length, 2.5 cm width and 0.5–3.5 cm wall thickness. Wood samples of *Fagus sylvatica* and *Pinus sylvestris* were cut to 5 cm length, 2.5 cm width and 1.5 cm height. Samples were put into a climate room at 20±2ºC and 65±5% relative humidity (RH) for 4 weeks, weighed, wrapped in plastic (Sengewald Flexopeel, Germany) and send for gamma radiation to BBF sterilization service GmbH (Kernen, Germany). To calculate the initial weight, moisture content at 20ºC and 65 % was adjusted downward to the oven-dry value.

Vermiculite test

The decay experiment was performed in 500 ml preserving jars (Fig. 1). Vermiculite of 2-3 mm particle size was obtained from vermiculite-shop (thinex new media, Dortmund, Germany, www.vermiculit-shop.de) and sieved through a 2 mm sieve. Each preserving jar was filled with 200 ml vermiculite. Tap water or 2% malt extract solution were added in 80, 100, 120, 140 and 160 ml amounts per jar. The jars covered with glass lids were sterilized in the autoclave at 121°C and 210 kPa for 30 min. Each two bamboo or wood samples were put aseptically in one jar. Two jars were used for one fungus. Vermiculite and sample were separated by a metal ring to avoid liquid diffusion in the sample. A plug of the test fungus from agar plates was then placed on the samples top. After 32 weeks of incubation at 20±2ºC and 65±5% RH, the mycelium was removed from the sample surface, the samples were weighed for final wet weight and oven dried at 103ºC for 3 days. Mass loss was calculated by weight comparison before and after incubation. Standard deviation for each 4 specimens per treatment group was calculated according to ANOVA, SPSS software 2002.



(a) (b)

Figure 1. Vermiculite test setup.
(a) bottle with vermiculite and bamboo samples. (b) incubation room.

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Assessment of fungal growth and vitality test after incubation

The development of fungal growth on the samples was assessed according to the scheme in table 1, which values hyphal density and hyphal coverage of sample. After 32 weeks of incubation, portions of overgrown vermiculite were transferred from the jars to malt agar plates to prove the vitality of the mycelium after the vermiculite test.

Table 1. Evaluation scheme for hyphal growth on bamboo and wood samples.

Hyphal density	Description	Hyphal coverage of sample	Description
0	No growth	0	No coverage
1	Sparse mycelium	1	1 - 33% coverage
2	Normal mycelium	2	34 - 66% coverage
3	Thick mycelium	3	67 - 99% coverage
		4	Total coverage

RESULTS AND DISCUSSION

Figure 2 shows details of fungal growth on bamboo samples and on vermiculite particles.

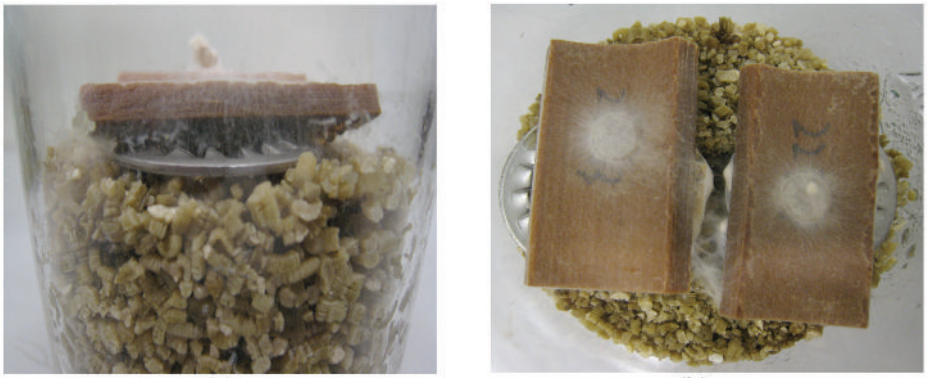

Figure 2. Details of a preserving jar with mycelium of *Schizophyllum commune* covering *Phyllostachys pubescens* samples.
(a) side view. (b) top view.

All bamboo and wood samples were totally covered by mycelium after 32 weeks (Table 2). Hyphal density with tap water was less than with malt extract. However, the density of mycelium is not necessarily related to mass loss in test specimens (Schmidt 2006).

Table 2. Fungal growth on bamboo and wood samples after 32 weeks incubation.

Bamboo	Fungus	Liquid	Liquid (ml) per preserving jar									
			80		100		120		140		160	
			D	C	D	C	D	C	D	C	D	C
Gigantochloa atroviolacea	*Coniophora puteana*	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
	Schizophyllum commune	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
Phyllostachys pubescens	*Coniophora puteana*	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
	Schizophyllum commune	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
Fagus sylvatica	*Schizophyllum commune*	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
Pinus sylvestris	*Coniophora puteana*	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	3	4	3	4	3	4	2	4	2	4

D = hyphal density, C = hyphal coverage of sample

Results of the degradation tests are summarized in figures 3 and 4. Standard deviation is not shown for the less mass loss by *S. commune* (Fig. 3), but is shown for *C. puteana* (Fig. 4) due to its greater decay capacity. Both bamboo species *Gigantochloa atroviolacea* and *Phyllostachys pubescens* were rather resistant to degradation by the white-rot fungus *S. commune* and the brown-rot fungus *C. puteana* over 32 weeks of incubation, the first bamboo being more resistant than the latter one. Adding different amounts (80 to 160 ml) of water or nutrient solution to vermiculite had only small effect. The final bamboo moisture contents were 61 to 84 %, which is a suitable range for decay fungi (Schmidt 2006).

Figure 3 shows the results for *S. commune.* Maximum mass loss of bamboo was only 4.6%. Thus, possible influences among the parameters, bamboo species, moisture content and nutrient addition, were rather indistinct for this fungus. Low decay was also measured on agar in preserving jars and in Kolle flasks (Schmidt *et al.* 2011) which was already reported by Suprapti (2010) and Kim *et al.* (2011). Abdurachim (1964) obtained 15% mass loss in *Gigantochloa apus*. Also beech wood showed neglectable decay (0.7-2 %) by *S. commune* (Fig. 3), which corresponds to previous results on low degradation of wood samples (Schmidt and Liese 1978). However, this fungus is common on bamboo culms during storage and use (Liese 1985, Mohanan 1997, Liese and Kumar 2003).

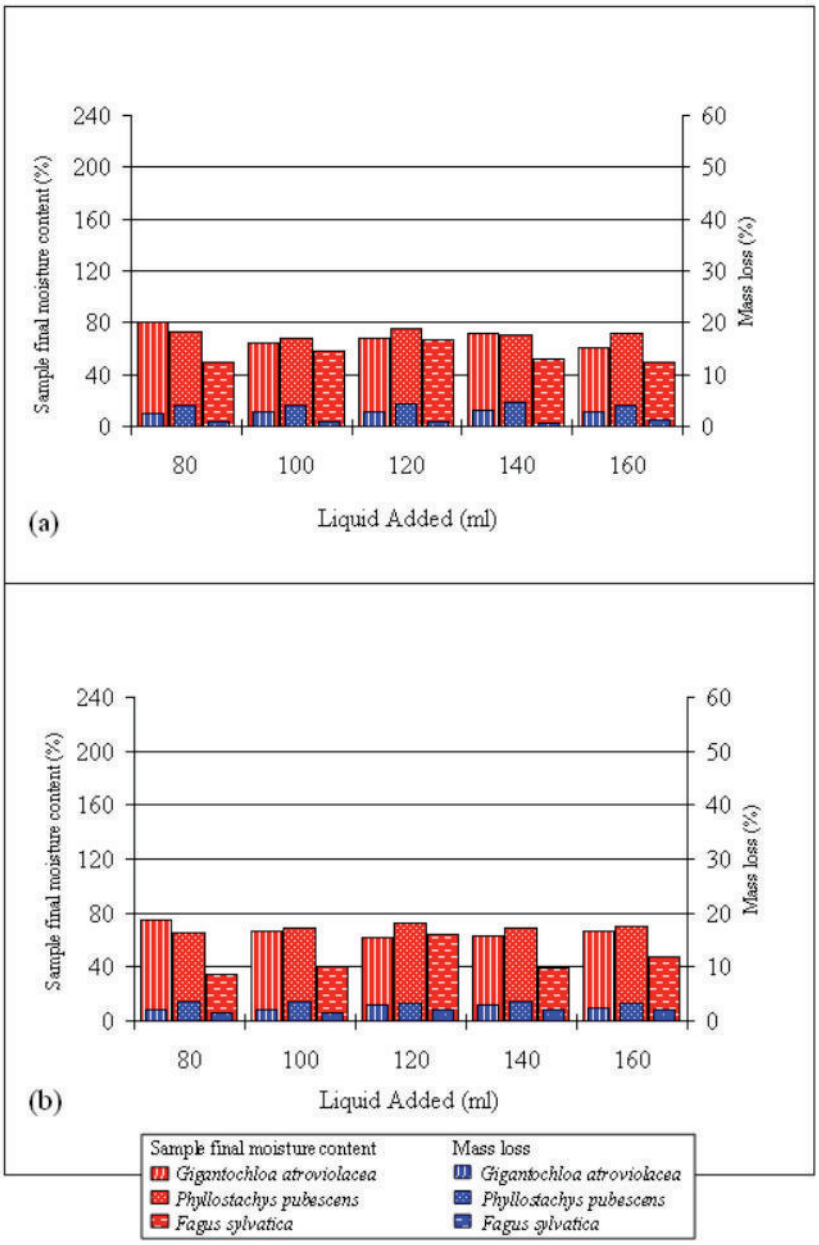

Figure 3. Influence of moisture content and nutrients on mass loss by *Schizophyllum commune.* (a) incubation with tap water. (b) incubation with malt extract solution.

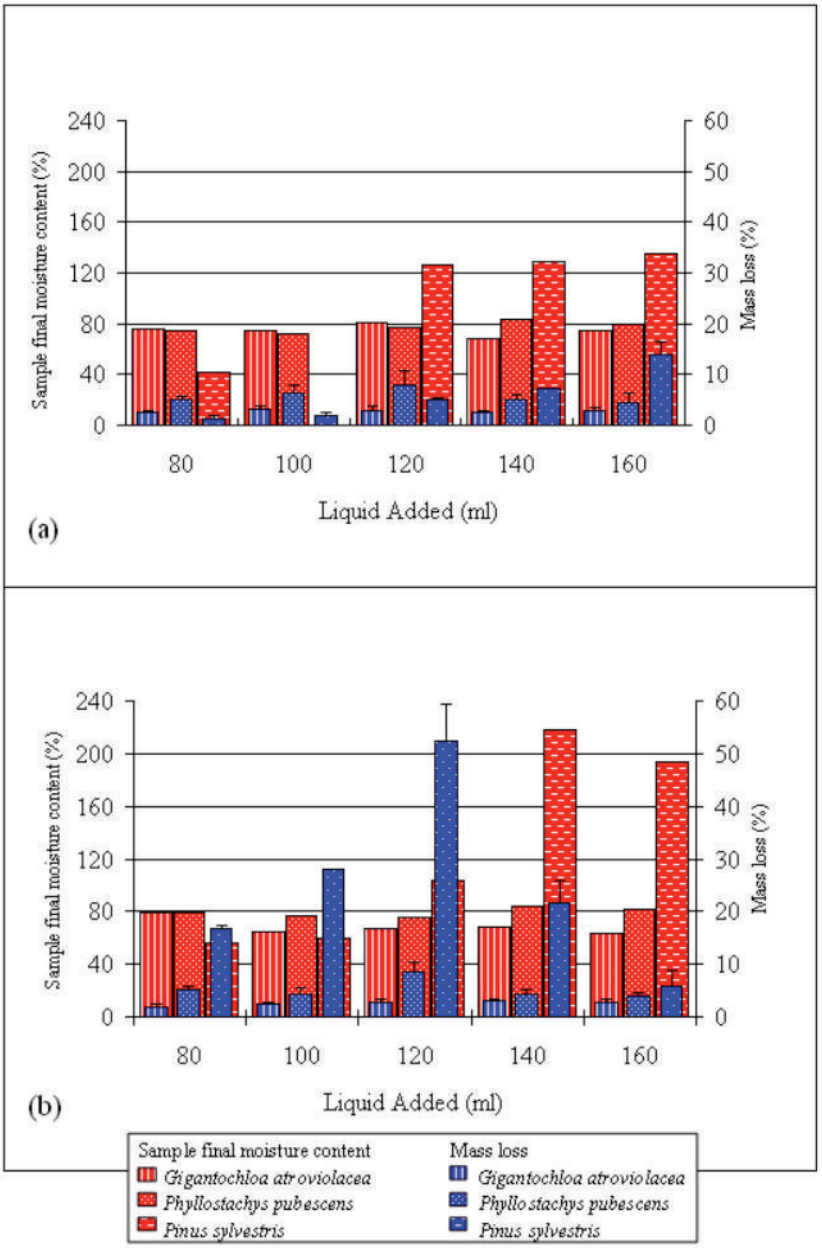

Figure 4. Influence of moisture content and nutrients on mass loss by *Coniophora puteana.* (a) incubation with tap water. (b) incubation with malt extract solution.

Figure 4 shows the results for *C. puteana.* Maximum mass loss of bamboo was 8.4%, whereby *P. pubescens* was more decayed than *G. gigantochloa.* Both liquid additions, tap water and malt extract, increased mass loss from 80- to 120 ml-addition followed by decrease to 160 ml. Scots pine wood revealed significant differences among the liquid additions: With tap water, decay increased stepwise to 14.0 % maximum at 160 ml water addition. Most decay with 52.5% maximum at 120 ml (104 % moisture content) occurred with the malt extract culture. The subsequent decrease of mass loss may be caused by too high moisture content.

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Fungi are able to transport water by their mycelia from a moisture source to neighbouring wood (Schmidt 2006). Figures 3 and 4 show that the final moisture content of the bamboo samples was not influenced by the different initial liquid additions. Obviously, both fungi did not transport water from vermiculite to the samples. Presence of the metal ring between vermiculite and sample could not be the reason because *C. puteana* could moisten the Scots pine wood. Figure 4 (bottom) shows that moisture content increased up to 218 %u parallel to water addition. Subsequently, mass loss decreased because of too much water in the wood.

The vitality test after 32 weeks of incubation proved living mycelium in all culture vessels (Fig. 5). Because fruit bodies were not grown in the jars, survival was not due to resistant spores. Thus, it can be deduced that mycelia were active over the whole culture period.

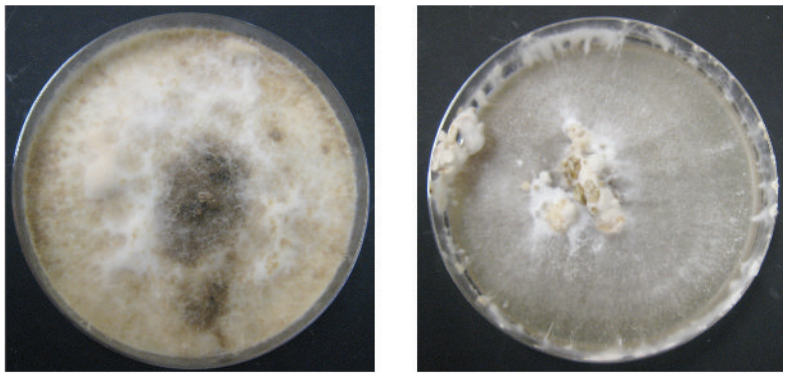

Figure 5. Vitality test on agar after vermiculite incubation.
(a) *Coniophora puteana.* (b) *Schizophyllum commune.*

Schmidt *et al.* (2011) assumed that high bamboo mass loss by *C. puteana* and *S. commune* in the fungus cellar test was mainly caused by the moisture conditions and lesser by components from the unsterile soil. The vermiculite test as pure-culture experiment in closed vessels did not show a significant effect of moisture content and nutrient addition on bamboo degradation. Thus, bamboo degradation in nature may be also influenced by better air conditions, soil minerals or vitamins from soil bacteria. Already Leutritz (1946) demonstrated that soil serves as water holding substrate and provides substances from the soil. The influence of minerals and vitamins on soft rot was reported by Worrall and Wang (1991) and Worrall *et al.* (1991). However, the small differences of mass loss among the bamboos, fungi and test parameters make further discussion problematic. The fungi had been selected due to three reasons: *S. commune* is very common on bamboo and the experimental strain was isolated by us from bamboo; *C. puteana* is an obligatory test fungus in the European standard EN 113; third, it was hoped to explain the influence of moisture content and nutrients in the fungus cellar test. However, with regard to wood, the decay results with *C. puteana* and *P. sylvestris* samples (Fig. 4) show the suitability of the proposed technique for degradation studies. Assumably, our vermiculite method will also show greater fungal activity against bamboo if more aggressive fungi such as *Pleurotus ostreatus* and *Trametes versicolor* (Schmidt *et al.* 2011) are used.

CONCLUSION

The preserving jar/vermiculite technique is suitable for fungal degradation studies of lignocelluloses as shown by the mass loss of *Pinus sylvestris* wood by *Coniophora puteana.* Adding 2% malt extract solution till wood moisture content around 100% provided high mass loss within 32 weeks of incubation. However, with regard to bamboo, interpretations are problematic due to the low activity of both fungi on bamboo.

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We thank Wolfgang Eberts (Bamboo Centre Germany) and Christoph Tönges (CONBAM, Germany) for providing the bamboo and Marie-Therese Lenz for technical help.

REFERENCES

Abdurachim, M.R.A. 1964. Bamboo preservation in Indonesia. *Rimba Indonesia* 9(1): 66-76.

Adaskaveg, J.E.; Blanchette, R.A.; Gilbertson, R.L. 1991. Decay of date palm wood by white-rot and brown-rot fungi. *Canadian Journal of Botany* 63(3):615-629.

Becher, G.; Kaune, P. 1966. Einflüsse beim Holzabbau durch Moderfäulepilze in Erde. *Material and Organisms* 1(3):201-220.

Curling, F.C.; Clausen, C.A.; Winandy, J.E. 2002. Experimental method to quantify progressive stages of decay of wood by basidiomycete fungi. *International Biodeterioration & Biodegradation* 49(1):13-19.

EN 113. 1996. Determination of the toxic values of wood preservatives against wood destroying Basidiomycetes cultured on agar medium. Beuth, Berlin.

Kaune, P. 1970. Bedingungen für das Prüfen mit Moderfäulepilzen im Vermiculit-Eingrabe-Verfahren. *Material and Organisms* 5(2):95-112.

Kim, J.J.; Lee, S.S.; Ra, J.B.; Lee, H.; Huh, N.; Kim, G.H. 2011. Fungi associated with bamboo and their decay capabilities. *Holzforschung* 65(2): 271-275.

Leutritz, J. 1946. A wood-soil contact culture technique for laboratory study of wood destroying fungi, wood decay and wood preservation. *Bell System Technical Journal* 25(1): 102-135.

Liese, W. 1959. Bamboo preservation and soft-rot. Food and Agriculture Organization Report to the Government of India, 1106, 36 pp.

Liese, W. 1985. Bamboos - biology, silvics, properties, utilization. Schriftenreihe Gesellschaft für Technische Zusammenarbeit, Eschborn, 180, 132 pp.

Liese, W.; Kumar, S. 2003. Bamboo preservation compendium. International Network for Bamboo and Rattan, Beijing, China, Technical report 22, 231 pp.

Ma, X.; Jiang, M.; Qin, D. 2010. The invasion channels of damage fungi in bamboo lumber. International Research Group on Wood Preservation, Stockholm, IRG/WG 10-10712, 7 pp.

Mohanan, C. 1997. *Diseases of bamboos in Asia.* International Development Research Centre, New Delhi.

Peylo, A. 2001. Schnellanalyse von Holzschutzmitteln. *Schützen & Erhalten.* 3p.

Schmidt, O. 2006. *Wood and tree fungi.* Springer, Berlin.

Schmidt, O.; Liese, W. 1978. Biological variations within *Schizophyllum commune. Material and Organisms* 13(3): 169-185.

Schmidt, O.; Wei, D.; Liese, W.; Wollenberg, E. 2011. Fungal degradation of bamboo samples. *Holzforschung* 65(6): 883-888.

Suprapti, S. 2010. Decay resistance of five Indonesian bamboo species against fungi. *Journal of Tropical Forest Science* 22(3): 287-294.

Worrall, J.J.; Wang, C.J.K. 1991. Importance and mobilization of nutrients in soft rot of wood. *Canadian Journal of Microbiology* 37(11): 864-868.

Worrall, J.J.; Anagnost, S.E.; Wang, C.J.K. 1991. Conditions for soft rot of wood. *Canadian Journal of Microbiology* 37(11): 869-874.