Olaf Schmidt
Wood and Tree Fungi
Biology, Damage, Protection, and Use

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With 74 Figures, 12 in Colors, and 49 Tables

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Foreword

Wood, as a raw material and a renewable biomass, has had great importance for thousands of years. Under suitable conditions, however, it is also easily degradable as part of the biological cycle. The processes of decomposition by fungi, and measures for protection against them, have been studied for quite a long time. The resulting knowledge on the causes and effects of wood degradation can hardly be overlooked.

For more than 30 years, Olaf Schmidt has investigated the causes and effects of wood degradation by fungi and bacteria. Pioneering contributions have also been made in several fields, such as wood-inhabiting bacteria and molecular methods for fungal identification. Laboratory work is accompanied by teaching the field of wood deterioration by microorganisms, thus contributing to the broad spectrum of information accumulated.

"Wood and Tree Fungi" by Olaf Schmidt presents the most comprehensive treatise on the fundamentals, causes, and consequences of decomposition of wood as well as measures for its prevention. The 1,400 references give an overlook of the vast amount of information evaluated. For a long time to come this book will be the competent source of knowledge about the fascinating interactions between wood and microorganisms.

Walter Liese

Preface

This book is the updated revision of the German edition "Holz- und Baumpilze" from 1994. Errors were corrected and new results were included. Particularly the chapter "Identification" was supplemented by molecular techniques. I realize that a one-author book on a relatively broad topic must include errors and also may have ignored recent literature. Strictly speaking, one should only write about things that they have experienced themselves, in the case of point this only concerns some aspects of bacteria and those fungi which inhabit the xylem of dead wood. Thus, current "secondary literature" was used for those chapters that are "on the edge" of my own research interest.

For better readability, the authors of fungal names are not mentioned in the text, but summarized in an appendix. Fungal synonyms are also not given. These are available from Index Fungorum (www.indexfungorum.org/names/names.asp). Fungal names cited from (older) publications were transferred to the current version.

Thanks for general advice go to Prof. Dr. Dr. h.c. mult. Walter Liese, for critical reading to Prof. Dr. Dirk Dujesiefken (Chap. 8.2), Dr. Hubert Willeitner, and Dr. Peter Jüngel (Chap. 7.4), to Mrs. Ute Moreth for providing experimental data, Dr. Tobias Huckfeldt for several photographs, many colleagues for permission to use their photographs, Mrs. Christina Waitkus for transferring many pictures in an electronic version, to Springer-Verlag, particularly Mrs. Ursula Gramm and Dr. D. Czeschlik, for co-operation, to Mr. Jardi Mullinax for making my English understandable, and to Mrs. Cornelia Gründer for careful printing.

Hamburg, December 2005 *Olaf Schmidt*

Contents

1 Introduction

Wood is damaged by various agents (Table 1.1).

This book addresses wood damage caused by microorganisms (fungi and bacteria). Wood damage by fungi has also been called "wood diseases" ("Holzkrankheiten") and "wood pathology" ("Holzpathologie"). Because it concerns the substrate "tree" in the majority of dead cells, because all parenchyma cells in the wood of felled trees are dead after a few weeks, and, thus, because a dead tissue cannot fall ill, distance was taken to these terms. With regard to the microbial decomposition of biomass, in the English language there is a well-describing differentiation between "biodeterioration", which means unwanted biological destruction, and "biodegradation", which means controlled degradation by microorganisms or their enzymes and degrading agents. Biodeterioration corresponds to the German "Holzzerstörung" and "Holzzersetzung", and the latter positive aspect of wood biodegradation ("Holzabbau") belongs to the area of "biotechnology of lignocelluloses" (Bruce and Palfreyman 1998; Chap. 9).

In the following text, the microbial damage to the xylem (wood) of the tree is mainly addressed. Since leaves, bark, and roots are entrance gates for parasites into the living tree that can lead to reduced tree growth and to lesser wood quality, some aspects of the area of "forest pathology" are included (Butin 1995; Chaps. 5 and 8.1–8.3). The mechanisms of the decomposition of solid

Table 1.1. Agents for wood damages

– mankind: e.g., paper production, fire for cooking
– conflagrations for agriculture
– weathering, UV light
– acids, bases, corrosion by salts, gases, discoloration by metals
– wood insects: xylophagous beetles, termites, wasps, breeding ambrosia beetles, wood-colonizing ants
– marine borers
– bacteria: wetwood, discoloration, pit degradation, decay by erosion, tunneling, cavity bacteria
– fungi: wood discoloration by molds, blue-stain fungi, red-streaking fungi wood decay by brown, soft, and white-rot fungi

wood apply essentially also to wood-based composites (plywood, fiberboard, particleboard, orientated strand board) (e.g., Chung et al. 1999) and to wood-plastic composites (Simonson et al. 2004). Sutter (2003) and Unger et al. (2001) report on damages, conservation, and restoration of wood artifacts. Bacterial and soft-rot attack of archaeological wood is described by Blanchette (1995), Nelson et al. (1995), and Singh et al. (2003).

The decomposition of biomass, which concerns wood and other lignocelluloses (annual plants), is a necessary part of the natural material cycle: during photosynthesis, wood and O_2 are formed from CO_2 and H_2O by means of light. In counterpart, the wood becomes degraded by fungi and bacteria to CO_2, H_2O and energy for microbial metabolism.

In the forests of the earth, about 400 billion t of CO_2 are bound. Without microbial degradation (or burning) of the biomass, the CO_2 supply of the atmosphere necessary for photosynthesis would be used up in 20–30 years (Schlegel 1992), photosynthesis would grind to a halt, and the earth would be overfilled with non-decaying biomass.

Humans retard wood degradation by microorganisms for economic reasons by wood protection measures (Willeitner and Liese 1992; Goodell et al. 2003; Müller 2005; Chap. 7.4) in order to prolong the use of the raw material wood. Thus, for example, the service life of a beech sleeper, which would amount to about 3 years without any protection, extends to about 45 years after impregnation with coal tar oil.

Until around 1800, rot was considered punishment from God, and fruit bodies as eczemas. Still, in 1850, v. Liebig attributed decay to a "slow burning". In 1874, Robert Hartig recognized the causality between pest and damage and is thus considered the father of forest and wood pathology (Merrill et al. 1975). The first pure culture of a wood-degrading fungus was succeeded to Brefeld (1881).

Research on wood deterioration is done worldwide. The global network for cooperation in forest and forest products research is the International Union of Forest Research Organizations (IUFRO), which was created in Eberswalde, Germany, in 1892, and has 15,000 scientists in almost 700 member organizations in over 110 countries. Current research results on wood damages, protection, and investigation methods are introduced at the annual symposia of the International Research Group on Wood Preservation (IRG). Edible mushrooms cultured on wood are discussed at the meetings of the International Mycological Society. A recent comprehensive treatise on the various aspects of fungi is "The Mycota" (Esser 1994 et seq.).

2 Biology

2.1 Cytology and Morphology

"Wood fungi" are eukaryotic and carbon-heterotrophic (free from chlorophyll) organisms with chitin in the cell wall, reproduce asexually and/or sexually by non-flagellate spores, filamentous, immovable and mostly land inhabiting. Damage to wood in water by fungi is described by Jones and Irvine (1971), Jones (1982) and Kim and Singh (2000). Soft-rot fungi belonging to the Ascomycetes and Deuteromycetes (Chap. 7.3) destroy wood with high moisture content in water or soil (e.g., Findlay and Savory 1954; Liese 1955). Fungi associated with leaf litter in a woodland stream were treated by Suberkropp (1997).

In this book, a fungal cell, the hypha, is defined as one individual cell of mostly tubular shape that consists of a cell wall, contains a protoplasm with a nucleus and other organelles, and is in the "higher fungi" separated from its one or two neighbors by a transverse wall, the septum (Fig. 2.1). In analogy to the "higher plants", where nearly every living cell is connected to its neighbors by cytoplasmic channels, the plasmodesmata, which pass through the intervening cell walls, also the protoplasms of neighbored hyphae are connected with each other through the pore or dolipore system (Fig. 2.2). This basic hypha is termed "vegetative hypha" in this book. This definition contrasts to others where one hypha, also termed generative hypha, is a more or less long filament consisting of several hyphal "compartments", a definition that is hazy because the transition to the next higher unit, the mycelium, is flowing. The mycelium is thus the filamentous lining up of hyphae, consisting in young mycelia of only a few vegetative hyphae and in older ones of several and branched hyphae. Figure 2.1 shows septate hyphae as they occur in the wood-inhabiting Deuteromycetes, Ascomycetes, and Basidiomycetes.

The diameter of hyphae reaches from 0.1–0.4 µm for the microhyphae of *Phellinus pini* (Liese and Schmid 1966) to 60 µm for the vessel hyphae in the mycelial strand (cord) of the True dry rot fungus, *Serpula lacrymans*, with an average for vegetative hyphae of about 2–7 µm (*S. lacrymans*: 3 µm: Seehann and v. Riebesell 1988). Their length reaches from about 5 µm for round/oval cells (spores) up to several micrometers. The size of many bacteria is between 0.4 and 5 µm.

Fig. 2.1. Vegetative hyphae. *C* coenocytic hyphae, *S* septate hyphae

Due to the smallness of the individual hypha and the use of microscopic and microbiological methods, fungi are microorganisms. This attachment does not contrast to the fact that fungi can form large and firm structures such as fruit bodies of decimeters in size like in the Tinder fungus, *Fomes fomentarius* (see Fig. 8.15). Those fruit bodies are, however, also composed of single hyphae. The main argument is, however, that the "actual fungus" is the vegetative hyphal system that can grow unlimited by simple mitotic reproduction without ever fruiting if fresh nutrients (wood, soil, agar) are available, and if growth in a certain biotope is not inhibited by the own or foreign metabolic products.

Fungi are scientifically examined in microbiological or medical institutes (predominantly Deuteromycetes and Ascomycetes) and often in botanical institutes. They do, however, no longer rank among the plants. In multi-kingdom systems (Whittaker 1969), the "higher fungi" (Ascomycetes, Basidiomycetes) form the distinct group of fungi beside the Prokaryotes (Bacteria), Protista (eukaryotic single-celled organisms: slime fungi and "lower fungi"), plants, and animals (Müller and Loeffler 1992). Based on rDNA sequences, Woese and Fox (1977) divided the Prokaryotes into the kingdoms Eubacteria and Archaebacteria and later emphasized three domains, which were renamed Bacteria, Archaea, and Eucarya (see Fig. 5.1).

The hyphal wall defines the shape of the hypha and provides the mechanical strength to resist the internal turgor pressure. The wall consists of various carbohydrates. Some yeast has mannan-β-glucans, while Ascomycetes, Deuteromycetes, and Basidiomycetes possess chitin-β-glucans, never cellulose. Chitin [poly–β(1-4)-*N*-acetoamido-2-deoxy-D-glucopyranose], which occurs except in fungi also in the exoskeleton of arthropods and crustaceans, and in some mollusks, is a macromolecule made of β-1,4-glycosidically linked *N*-acetylglucosamine units. Chitin synthases (CHS; EC 2.4.1.16) catalyze the formation of chitin from the precursor UDP-*N*-acetylglucosamine. In the yeast *Saccharomyces cerevisiae*, CHS I acts as a repair enzyme and is involved in the chitin synthesis at the point where the daughter and mother cells separate. CHS II participates in septa formation and CHS III in chitin synthesis of the cell wall (Robson 1999). Ascomycetes have two-layered cell walls, while walls of Basidiomycetes are multilamellar. The entire structure of the cell wall including extracellular layers is complex (Toft 1992; Robson 1999): The wall of filamentous fungi may consist for example of an inner wall of about 10–20 nm composed of chitin microfibrils and an outer wall composed of a protein layer (about

10 nm), a layer of glycoprotein (about 50 nm), and a slime layer, also termed mucilage layer, sheath, extracellular matrix or mycofibrils (about 75–100 nm).

Slime layers are common to fungi and have been found in blue stain, white, brown, and soft-rot fungi. They are composed of protein, lipid and carbohydrate containing material (α-glucan, β-1,3 and β-1,6-glucan) or of crystalline to membranous and fibrillar structures (Liese and Schmid 1963; Schmid and Liese 1965; Schmid and Baldermann 1967; Holdenrieder 1982; Green et al. 1989). Various functions have been suggested for the slime layer (Schmid and Liese 1966; Sutter et al. 1984; Green et al. 1991b; Kim 1991; Abu Ali et al. 1997; Messner et al. 2003; Table 2.1). In *Phanerochaete chrysosporium*, the slime layer is composed of equal amounts of carbohydrates, lipids, and proteins, including five fractions with molecular weights between 30 and 200 kDa (cf. Messner et al. 2003). Production of the slime layer was influenced by iron, manganese and nitrogen concentration, temperature, and pH value (Jellison et al. 1997).

Hyphae may be encrusted and covered with resinous material, oil drops, and calcium oxalate crystals (e.g., Holdenrieder 1982).

The hyphal wall encloses the cytoplasm with its outer boundary, the plasmalemma. In the majority of fungi, ergosterol is the chief sterol in the plasma membrane and is used for fungal quantification (Chap. 2.4). Some antifungals like polyene and triazole act on this ergosterol (Robson 1999). The cytoplasm principally resembles that one of plants. There is one too many relatively small nuclei. Plastides are absent. Growing hyphae of Ascomycetes and Basidiomycetes show at the hyphal apex a mass of small vesicles, the "Spitzenkörper". The tonoplast encloses a vacuolar system. Carbon is stored in glycogen vesicles and lipid vacuoles. Nitrogen is deposited as amino acids in the vacuolar system or as protein. Phosphorus is condensed as polyphosphate in volutin grana, often in vacuoles. Some yeast contains starch.

Table 2.1. Possible functions for fungal slime layers

– substrate recognition
– adhesion to and establishing contact
– covering the S_3 layer of the wood cell wall during decay process
– conditioning of the substrate for decay
– modification of the extracellular ionic environment and pH-value
– transport vector for low-molecular decay agents and enzymes to the wood (see Fig. 7.3)
– transport vector for degradation products to the hypha
– storage, concentration and retention of decay agents
– regulation of the decay process, e.g., by controlling the glucose level
– microenvironment for H_2O_2 maintenance needed for lignin degradation
– storage of nutrients
– permitting a film of liquid water to surround the wood cell wall
– protection of the mycelium against dehydration and adverse environmental conditions
– increase of surface area for aerobic respiration
– storage of copper or CCA from attack of impregnated wood

The solutes in the cytoplasm and vacuolar system have a certain osmotic potential. If the potential is lower than that of the substrate, water is adsorbed across the membranes, increasing the volume of the cytoplasm (Jennings and Lysek 1999). An internal turgor needs to be generated for the elongation of the hyphal apex that is that water uptake and wall growth are in balance.

Mycelium is the filamentous, partly branched, and in the wood-inhabiting Basidiomycetes usually whitish network made from some to numerous, in the light microscope hyaline to light yellow and in the case of blue-stain fungi brownish (melanin) hyphae. In Deuteromycetes, the pigmentation of the culture is manifold due to the various pigments of the conidia, whose color depends on the species. Mycelium forms the macroscopically visible thallus, the undifferentiated form of vegetative growth of fungi (thallobionts), which is not differentiated as it is the kormus of the "higher plants" into the organs, shoot axis, leaf, and root. Mycelium is the actual fungus with nourishing function and thus with wood decay ability. Under sufficient nutrient offer, mycelium is theoretically growable for an unlimited period. Sexuality with fruit body formation is not necessary for survival. For example, mycelium of an isolate of the Asian edible mushroom Shiitake, *Lentinula edodes*, is now maintained since about 1940 exclusively on agar in the refrigerator without ever fructifying, but would immediately develop fruit bodies (Fig. 9.1) when favorable environmental conditions are provided (Schmidt 1990). The largest and longest-living wood fungi are *Armillaria* species. A clone of *A. gallica* in a Michigan forest covered 15 ha and was estimated at an age of about 1,500 years and a total biomass of 1,000 t (Smith et al. 1992). A clone of *A. ostoyae* estimated at 400–1,000 years covered an area of 6 km^2 in the Rocky Mountains (Anonymous 1992a). In Oregon, an *A. ostoyae* clone of 2,400 years stretched over an area of about 9 km^2 of forest soil (Schwarze and Ferner 2003).

Deutero- and Ascomycetes have in the septum a central simple pore. Wood-inhabiting Basidiomycetes (Homobasidiomycetes) contain the more complicated dolipore septum with a parenthesome on both sides (Fig. 2.2).

The protoplasts of neighboring hyphae are connected through pores in the septa for the longitudinal migration of organelles and even nuclei, and for the transport of solutes (translocation; Chap. 3.1). Woronin bodies, which are composed of protein, block the pore when a hypha becomes injured.

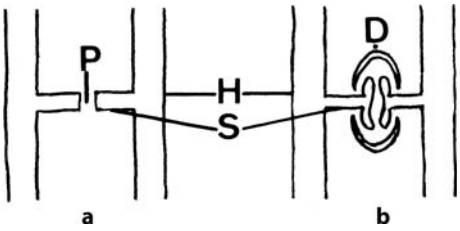

Fig. 2.2. Septa (*S*) of Ascomycetes (**a**) and Basidiomycetes (**b**). *P* simple pore septum, *D* dolipore septum, *H* hyphae

The hyphal system expands by extension of individual hyphae that exhibit apical growth and by branching (Fig. 2.3).

Different zones occur in the growing hypha (Fig. 2.4), which correspond to different ages and developmental stages (Huckfeldt 2003).

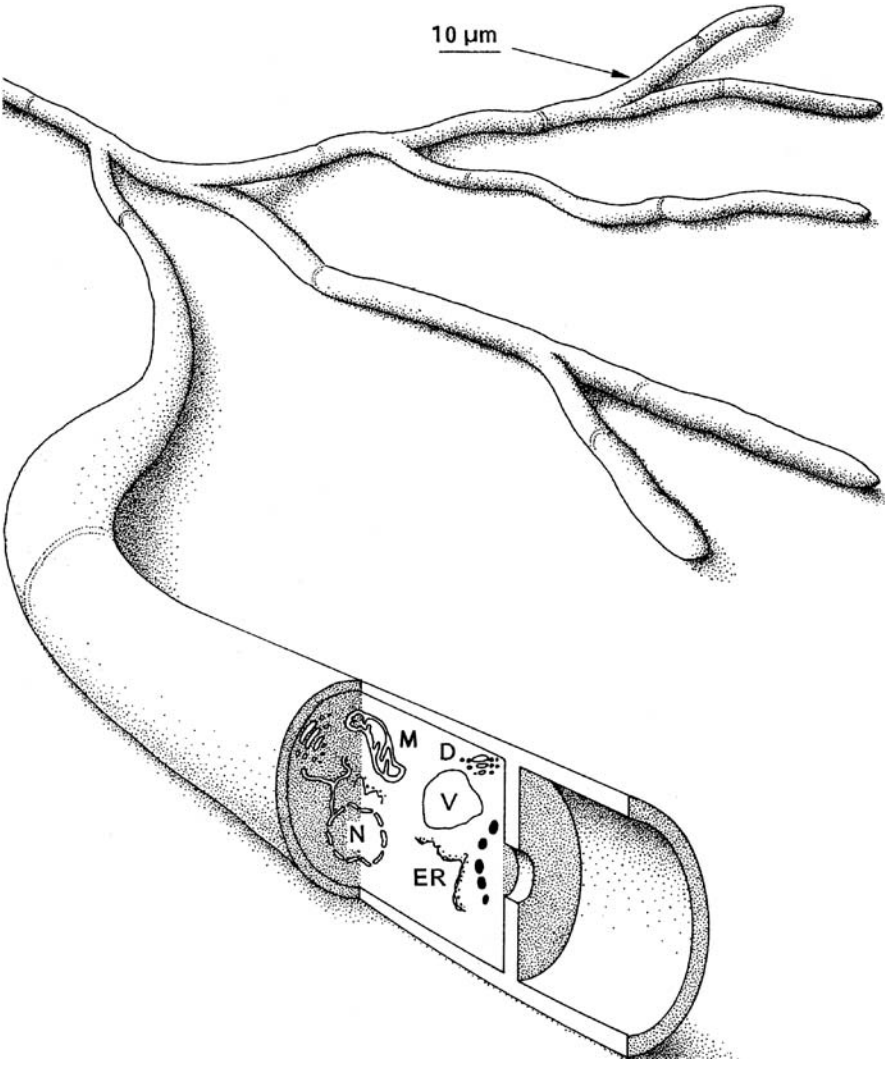

Fig. 2.3. Apical growth and hyphal branching system. One branch is sectioned to show the septum and some features of the protoplasm. *N* nucleus, *ER* endoplasmic reticulum, *D* dictyosome, *V* vacuole, *M* mitochondrion, Woronin bodies (*dark*) [reproduction with permission, from Jennings DH, Lysek G (1999) Fungal Biology, 2nd edn. Bios, Oxford, Fig. 1.1. page 6]

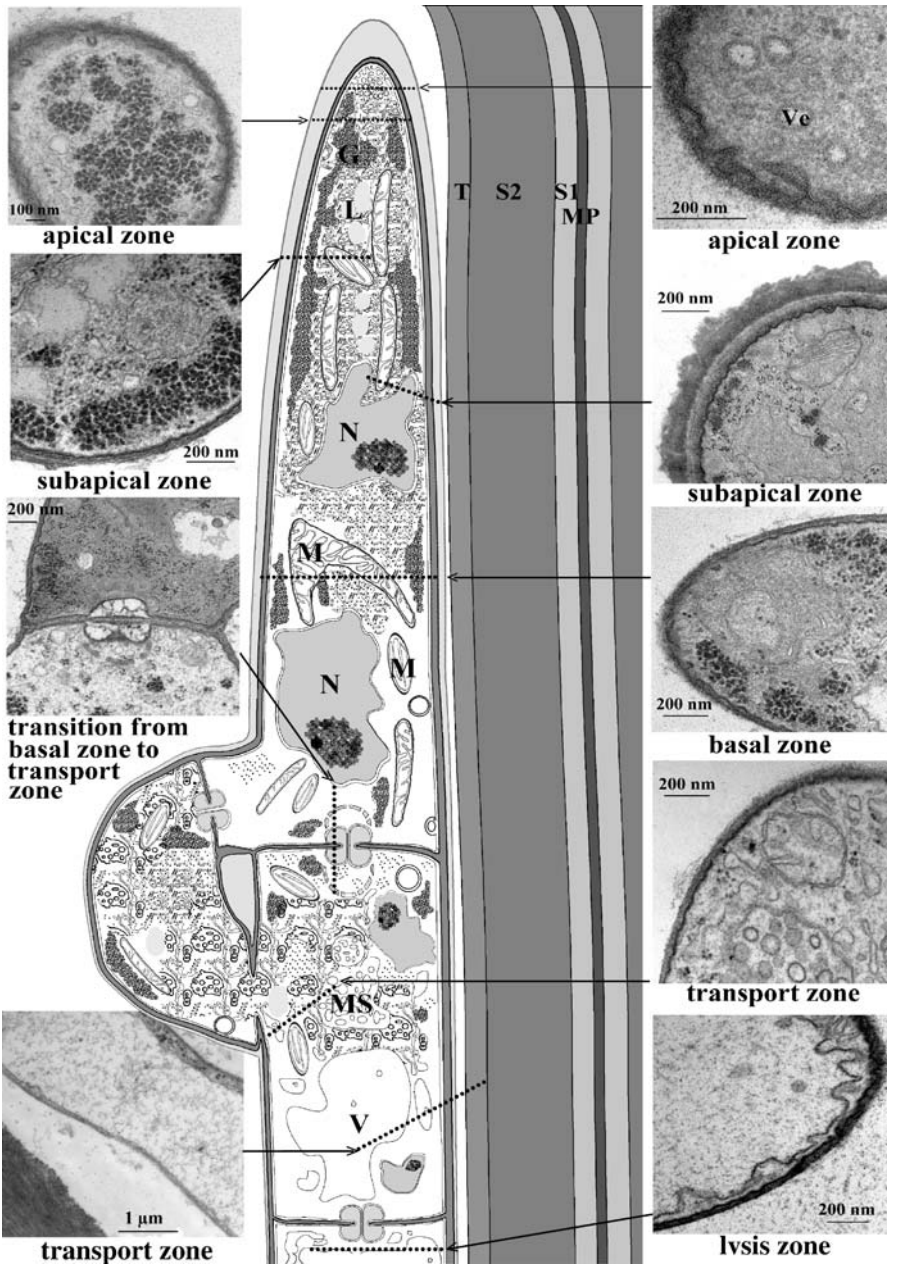

Fig. 2.4. Ultrastructural features and zonation of growing hyphae of a house-rot fungus in woody tissue (*MP*, *S*1, *S*2 and *T* wood cell wall layers). *G* glycogen, *N* nucleus, *L* lipid drops, *Mi* mitochondrion, *MS* multivesicles structure, *V* vacuole, *Ve* vesicle (from Huckfeldt 2003)

Due to the apical growth, the hyphal tip is the most sensitive part of the mycelium and gets the first contact with the substrate wood. Wood preservatives, unfavorable temperatures, shortage of nutrients, and moisture affect the tip. The tip contains different vesicles and membranous structures for cell wall synthesis and transport processes as well as enzymes for nutrient metabolism (Robson 1999). Like in other Basidiomycetes, the tip in *Serpula lacrymans* (Fig. 2.4) consists of three zones:

In the apical zone, material for the structure of the cell wall, slime layer, and plasmalemma is concentrated and incorporated in the growing mycelium. Vesicles from the Spitzenkörper merge with the plasmalemma and deliver cell wall components. In the subapical zone, compartments and ribosomes are involved in the synthesis of cell wall material and secretion products. The basal zone contains the nucleus, or in the case of dikaryons, two nuclei. Vacuoles are involved in internal recycling processes, detoxification, storage, upkeep of turgor pressure, control of ionic strength as well as metabolization of compartments and macromolecules. The cytoskeleton, which consists of actin filaments and microtubuli, serves together with motor proteins to the upkeep of the zonation of the hyphal tip by changing the position of the compartments. Thus, the compartments continuously follow the growing tip to maintain the density of organelles in the subapical zone. Jennings and Lysek (1999) differentiated the apical growth zone with the extending hyphal tip, the absorption zone where there is uptake of nutrients, the storage zone in which nutrients are stored as reserve substances, and the senescence zone where dark pigments and lysis may occur.

The hyphal system produces a loose network of filaments (aerial mycelium on the wood surface, substrate mycelium within wood and soil) or solid, morphologically differentiated units such as the strands of house-rot fungi and the rhizomorphs of *Armillaria* species (Chap. 2.2.1), and the fruit bodies.

The mycelia of wood fungi differ considerably in their growth rate. Table 2.2 shows the growth rate of some house-rot fungi.

The growth rate serves as a characteristic for species identification in keys. Growth rate is also used as a hint of the age of the fungal infestation time of a building, e.g., in the case of damage by *Serpula lacrymans* (Chap. 8.5.3.4). However, mycelial extension depends on environmental conditions like temperature and nutrients, which differ between stable and favorable laboratory conditions and fluctuations in buildings. Furthermore, different isolates of a species commonly differ in growth rate ("strain variation"). In addition, dikaryons and monokaryons may differ in growth. For example, dikaryons of *Lentinula edodes* (Schmidt and Kebernik 1987), *Serpula lacrymans* (Schmidt and Moreth-Kebernik 1991a), and *Stereum hirsutum* (Rayner and Boddy 1988) grew faster than the monokaryons. Nevertheless, there are so-called "fast-growing" wood fungi like the Cellar fungus, *Coniophora puteana*, with up to

Table 2.2. Growth rate of house-rot fungi at optimum temperature (from Schmidt and Huckfeldt 2005)

Group	Species	Number of investigated isolates	Maximum radial increase on agar per day (mm)
Dry-rot fungi	*Serpula lacrymans*	2	4.0 – 5.1
	S. himantioides	2	7.0 – 11.0
	Leucogyrophana mollusca	6	1.0 – 3.3
	L. pinastri	4	2.4 – 4.2
	Meruliporia incrassata	2	2.8 – 3.2
Cellar fungi	*Coniophora puteana*	27	2.5 – 11.3
	C. marmorata	2	9.7 – 12.3
	C. arida	1	4.7
	C. olivacea	5	3.7 – 9.0
White polypores	*Antrodia vaillantii*	12	4.3 – 7.7
	A. sinuosa	4	4.0 – 8.0
	A. xantha	3	5.5 – 8.2
	A. serialis	3	3.5 – 3.9
	Oligoporus placenta	4	4.2 – 9.8
Gill polypores	*Gloeophyllum abietinum*	5	3.8 – 5.5
	G. sepiarium	4	6.8 – 8.3
	G. trabeum	5	7.1 – 9.1
Oak polypore	*Donkioporia expansa*	1	5.1

11 mm radial increment per day on 2% malt extract agar at 23 °C and "slow-growing" species like *S. lacrymans* with up to 5 mm at 19 °C.

Mycelium of wood-decay fungi predominantly grows as substrate mycelium inside of the substrates wood (or soil) and is often not visibly on the outside, thus, wood rot, particularly at incipient decay, is frequently not recognizable outwardly. By means of surface mycelium, growth additionally or predominantly occurs on the substrate surface, e.g., on nutrient agar or in the case of molds that grow superficially on timber and masonry. Aerial mycelium, e.g., in the white polypores in buildings (*Antrodia* spp.), is an intensively developed surface mycelium. The texture of the mycelial mat is manifold, e.g., flat on the substrate, crusty, woolly, felty, or zonate (Stalpers 1978).

2.2 Growth and Spreading

2.2.1 Vegetative Growth

Simplistically, wood fungi live through two functionally different phases: the vegetative stage for mycelial spread and the reproductive stage for the elaboration of spore-producing structures. Rayner et al. (1985) extended the de-

velopment of a fungus in arrival, establishment, exploitation, and exit. The vegetative, asexual stage consists in wood fungi of vegetative hyphae with some specialized forms. The reproductive stage can both occur asexually or sexually (Schwantes 1996; Table 2.3).

Functional specialization of the mycelium occurs during the vegetative stage: germination, infection, spread, and survival. These functions are correlated with different "fungal organs". Spores (conidia, chlamydospores, also the sexually derived asco- and basidiospores) germinate under suitable conditions (moisture, temperature). The young germ hypha first shows some nuclei before the young mycelium grows with septation in the monokaryotic condition.

Mycelial growth takes place via mitoses and synthesis of hyphal biomass. Infection and colonization of new substrates occurs by spores, hyphae, mycelium, and special forms like bore-hyphae, transpressoria, strands, and rhizomorphs. Prerequisites for the colonization of a substrate are suitable humidity and nutrient availability in the substrate or, like in *Serpula lacrymans*, the ability of a fungus to transport nutrients and water and last, whether and by which organisms the substrate is already occupied (Rayner and Boddy 1988). Boring microhyphae of 0.1–0.4 µm diameter develop e.g., in *Phellinus pini* at the hyphal tip without recognizable septum and produce boreholes of 0.3–3.3 µm diameter probably by enzyme action (Schmid and Liese 1966). The appressorium is a hypha for the mechanical fixation to the substrate (Fig. 2.5a). The transpressorium (Fig. 2.5b) of the blue-stain fungi (Chap. 6.2) is a specialized boring hypha (Liese 1970); it is still unknown whether the penetration of the woody cell wall is by mechanical and/or enzymatic action. Transpressoria have also been found in the white-rot fungus *Phellinus pini* (Liese and Schmid 1966).

Table 2.3. Functional and morphological differentiation of wood fungi (modified after Müller and Loeffler 1992)

Developmental stage	Function	"Organ"
Vegetative/asexual	Germination	Germ hypha
	Infection, spread	Hypha, mycelium, boring hypha, appressorium, transpressorium, strand, rhizomorph
	Survival	Chlamydospore, arthrospore, mycelia with resistance to dryness and heat
Reproductive/asexual	Anamorphic reproduction	Fruit body, conidiophore, conidium
Reproductive/sexual	Teleomorphic reproduction	Fruit body, ascus, basidium ascospore, basidiospore

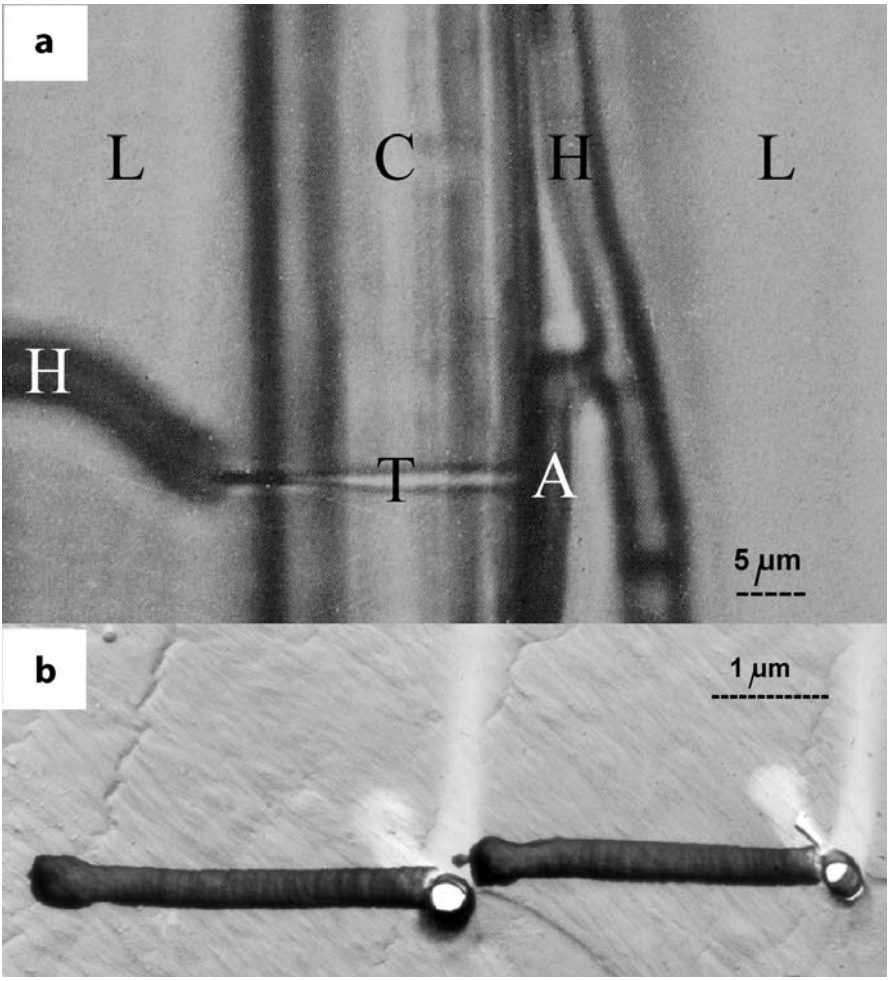

Fig. 2.5. Appressorium and transpressoria of blue-stain fungi in wood. **a** Hyphae (*H*) of *Ophiostoma piceae* in the luminina (*L*) of a pine tracheid. *A* appressorium, *T* boring canal through the activity of a transpressorium, *C* wood cell wall. (LM, from Liese and Schmid 1962); **b** Two transpressoria (EM, from Liese and Schmid 1966)

Strands (cords) (Fig. 2.6) develop in a number of house-rot fungi and usually consist of three hyphal types, vegetative hyphae, thin fiber (skeletal) hyphae with mostly thick walls for strengthening, and broad vessel hyphae for nutrient transport (Nuss et al. 1991). These hyphae form a distinct mycelium in the longitudinal direction, which is, however, not so well organized like the tissue-like structure of the rhizomorphs. Also in contrast to rhizomorphs, strands develop behind the mycelial growth front. Particularly *Serpula lacrymans* overgrows larger distances of non-woody substrates and penetrates through masonry

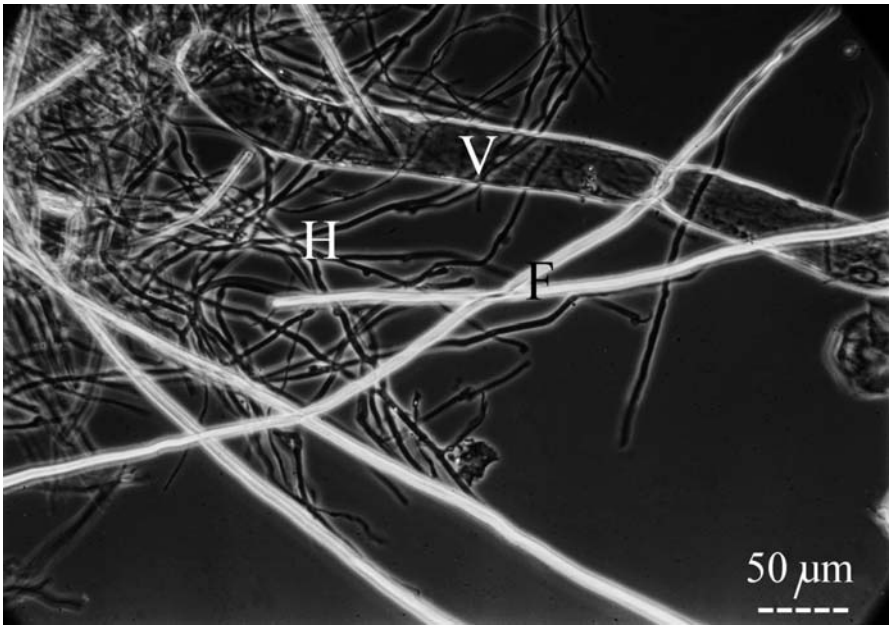

Fig. 2.6. Hyphae within a strand of *Serpula lacrymans*. *H* vegetative hyphae, *V* vessel hypha, *F* fiber hyphae (dark-field photo W. Liese)

(only through the joints) between bricks or through old, crumbly bricks, and insulation materials. In the laboratory, some house-rot fungi overgrew by means of strands agar that contained wood preservatives (Liese and Schmidt 1976) as well the fungal partner in dual culture.

In the literature, there is however not always a uniform use of the terms "strands (= cords)" and "rhizomorphs". For example, the strands of the American dry rot fungus, *Meruliporia incrassata*, have been termed rhizomorphs and were described as consisting of more or less parallel hyphae, outer (cortical) hyphae thick-walled and uniform in size (author: = fibers), inner (medullary) thin-walled hyphae, variable in size, and some differentiated into large conducting tubes (author: = vessels) (Palmer and Eslyn 1980). According to Burdsall (1991) "these two (*S. lacrymans*, *M. incrassata*) being similar and unique in forming large water-conducting rhizomorphs".

By means of his strand diagnosis, Falck (1912) was able to differentiate some house-rot fungi like *S. lacrymans*, *Coniophora puteana,* and *Antrodia vaillantii* macroscopically and microscopically. Table 2.4 shows an updated version for the above tree species based on recent measurements of mycelia in buildings and on agar cultures of genetically verified isolates.

As strand morphology is, after fruit body structure, a main feature to identify fungi growing indoors or an construction wood, an identification key for about

Table 2.4. Strand diagnosis for some common house-rot fungi (modified from Huckfeldt and Schmidt 2004, 2006)

Serpula lacrymans
Strands white, silver-grey to brown, more than 5 mm to 3 cm diameter, separable, with flabby mycelium in between, thick strands when dry breaking with clearly audible cracking (strands with mold contamination often not cracking any more), often in masonry; (*S. himantioides*: strands thinner than 2 mm and fibers 2 – 3.5 µm in diameter)
Vegetative hyphae hyaline, partly yellowish, with large clamp connections, 2 – 4 µm in diameter
Vessels at least partly numerous (in groups), 5 – 60 µm in diameter, not or rarely branched, with bar thickening up to 13 µm high
Fibers refractive, 3 – 5 µm diameter, straight-lined, stiff, septa not visible, no clamps, lumens often visible
Coniophora puteana, *C. marmorata*
Strands first bright, then brown to black, up to 2 mm wide, to 1 mm thick, root-like, hardly removable (not so in *C. marmorata*), when removed usually fragile, partly with brighter center, underlying wood becoming black, also in masonry
Vegetative hyphae usually without clamps, rarely multiple clamps (often indistinct when branched), 2 – 6 µm in diameter
Vessels surrounded and interwoven by many fine hyphae (0.5 – 1.5 µm in diameter), difficult to isolate (preparation with KOH solution); drop-shaped, hyaline to brownish secretions (1 – 5 µm in diameter) often on hyphae; vessels due to preparation irregularly formed or distorted, up to 30 µm in diameter, thin-walled (slightly thick-walled with *C. marmorata*), without bars, with septa
Fibers pale to dark brown, 2 – 4 µm in diameter, somewhat thick-walled, with relatively broad, usually visible lumen, some also branched, to be confused with generative hyphae
Antrodia vaillantii, *A. serialis*, *A. sinuosa*, *A. xantha*
Strands white to cream, partly somewhat yellowing or infected by molds, also ice flower-like, flexible also when dry, up to 7 mm in diameter, possibly also within masonry
Vegetative hyphae with few clamps, 2 – 4 µm in diameter, often somewhat thick-walled; in KOH somewhat swelling
Vessels not rare but in old strands difficult to isolate, up to 25 µm in diameter, thick-walled with middle lumen, without bars
Fibers hyaline (in *Antrodia xantha* partly somewhat yellowish), numerous, 2 – 4 µm in diameter, hyphal tips with tapering ending cell walls, straight-lined, mostly unbranched, insoluble in KOH, but partly somewhat swelling, then with "blown up" hyphal segments

20 strand-forming wood decay fungi based on Huckfeldt and Schmidt (2004, 2006) is given in Appendix 1.

The rhizomorphs of *Armillaria mellea* (Fig. 2.7) are tissue-like mycelial bundles with apical growth and consist of a black, gelatinous bark layer, followed by a pseudoparenchyma, and a central, loosely interwoven pith with vessel and

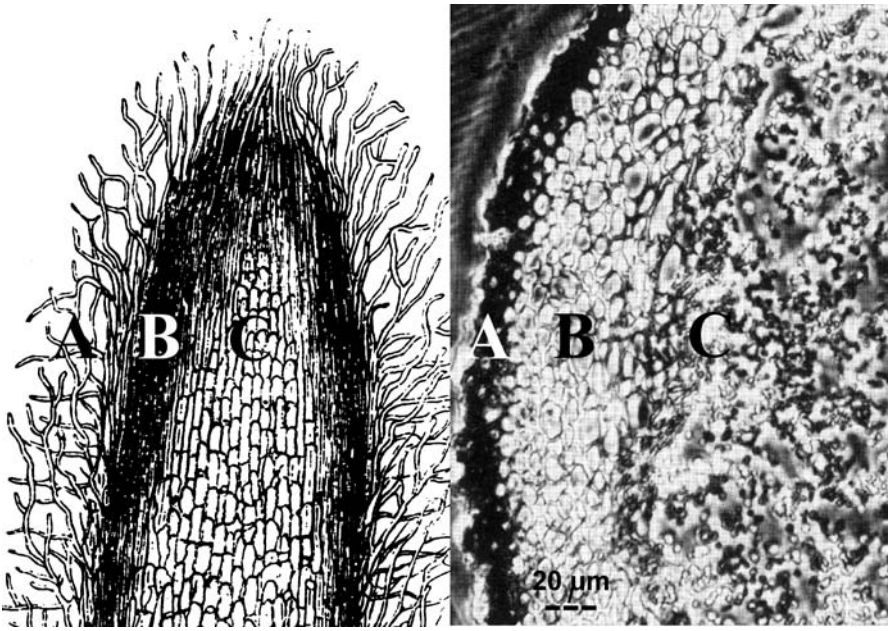

Fig. 2.7. Rhizomorph of *Armillaria mellea*. *Left*: Apex with hair-like microhyphae (*A*), cortex (*B*) and pith (*C*). (from Hartig 1882); *right*: cross section (LM; from Schmid and Liese 1970)

fiber hyphae (Hartig 1882). By means of rhizomorphs, *Armillaria* species grow in the soil and infect the roots of living trees (Chap. 8.3.1).

Under unfavorable conditions, resistance stages are formed. Spores are more resistant to heat, dryness, and wood preservatives than their mycelium. The hyphal cell water content is reduced, nutrients are concentrated, parts of the protoplasts or storage substances of neighboring cells are translocated in resting cells, and enzyme activity decreases ("latent life"). Chlamydospores (Fig. 2.8) are thick-walled spores with a brown cell wall, which occur in many blue-stain fungi.

Formerly, it was believed that the vegetative mycelium of some wood-decay fungi is also resistant to dryness (Chap. 3.3) and heat (Chap. 3.4). Recent results show that this must not be true: When cultured on agar at about 28 °C, the dikaryotic hyphae of *Serpula lacrymans* tend to revert to the monokaryotic condition, which regularly shows abundant arthrospores (Schmidt and Moreth-Kebernik 1990). In wood samples that were slowly dried or warmed, the substrate mycelium of *S. lacrymans*, *C. puteana*, *Donkioporia expansa,* and *Gloeophyllum trabeum* also formed arthrospores (Huckfeldt 2003). It was therefore assumed that these arthrospores are the agents for resistance against drying and heat.

2.2.2 Reproduction of Deuteromycetes

Fungi that reproduce asexually (anamorphic fungi) are either yeasts or Deuteromycetes. The term "yeast" is descriptive and stands for any fungus that reproduces by budding.

Deuteromycetes (Fungi imperfecti, colloquially: molds) is an artificial assemblage of fungi that reproduce asexually by conidia (conidiospores), either as the only form for propagation (imperfect fungi) or additionally (anamorph) to a sexual reproduction (teleomorph). When both the anamorph and the teleomorph are known, the fungus is called a holomorph (the whole fungus). The teleomorph may have one (mono-anamorphic) or many (pleo-anamorphic) asexual stages. In other words: Deuteromycetes are the conidia-producing forms of a fungus and may or may not be associated with a teleomorph. Many Deuteromycetes are supposed to have a teleomorph in the Ascomycetes, but they may also have basidiomycetous affinity. Also in the wood-inhabiting Deuteromycetes, the teleomorph often is of ascomycetous affinity as in the blue stain and soft-rot fungi, but some are anamorphs of Basidiomycetes like in the Root-rot fungus, *Heterobasidion annosum* [anamorph: *Spiniger meineckellus* (A.J. Olson) Stalp.; e.g., Holdenrieder 1989]. In the absence of a teleomorph, taxonomic affinity can be detected by the ultrastructure of the cell wall: Ascomycetes have two-layered walls, while the walls of Basidiomycetes are multilamellar. In terms of strict nomenclature, the teleomorph name takes precedence over the anamorph but in practice, a species is often identified according to the form in which it was found (Eaton and Hale 1993), like in the case of the wood-inhabiting molds *Aspergillus* and *Penicillium*.

The Deuteromycetes are usually divided in Coelomycetes and Hyphomycetes. Coelomycetes develop conidiophores within fruit bodies (conidiomata). In Hyphomycetes (or Moniliales), conidia develop on simple or aggregated hyphae. Conidium formation and conidiophore morphology are criteria to classify Deuteromycetes (Chap. 2.5). A simplified differentiation for wood-inhabiting Deuteromycetes (Fig. 2.8) distinguishes between conidiospore (free cell fragmentation at the hyphal tip or a branch) and sporangiospore (development in a sporangium).

Conidia of wood-inhabiting Deuteromycetes can be defined as mitotically developed (mitospores), immovable, mononuclear to more-nuclear, unicellular to more-celled, pigmentless (hyaline) to white, yellow, orange, red, green, brown, blue, or black colored (depending on the species) spores of different development, size, shape and surface (Fig. 2.9; Reiß 1997; Kiffer and Morelet 2000). The variety of the spore pigments causes that molded substrates may be colorful.

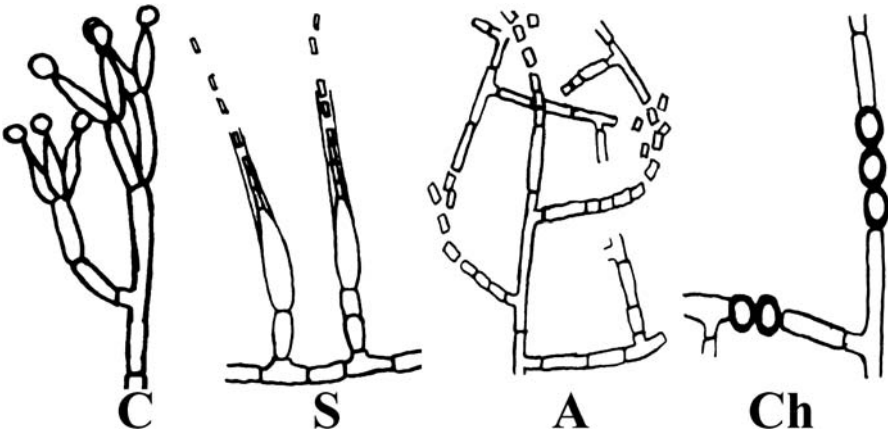

Fig. 2.8. Generalized view of conidia according to their development. *C* conidia, *S* sporangiospores, *A* arthrospores, *Ch* chlamydospores

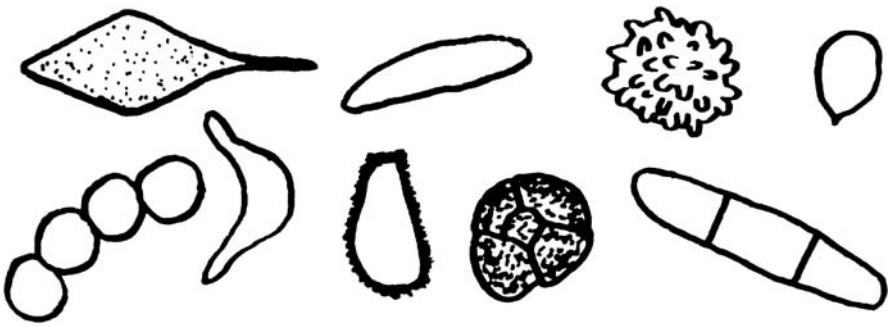

Fig. 2.9. Conidia. Example of the manifold shapes and structures

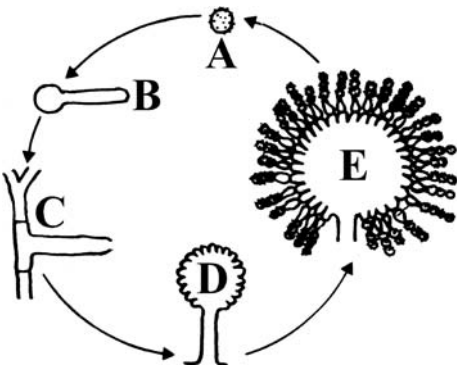

Fig. 2.10. Developmental cycle of a deuteromycete. *A* conidium, *B* germ hypha, *C* development of conidiophore, *D* development of vesicle, *E* vesicle with conidia

The series of spore germination, hyphal growth, and conidia production represents the asexual reproduction cycle of a deuteromycete fungus, illustrated in Fig. 2.10 by an *Aspergillus* species.

The biological advantage of the conidia production to the Deuteromycetes (and anamorphs of Asco- and Basidiomycetes) is that these fungi can exit from an exploited substrate to arrive fresh nutrients by spores (mitospores) in huge numbers without the need of preceding sexuality. Distributed randomly by and through the air or by adhering to the surface of animals, spores are present everywhere. Disadvantageous is that without (para)sexuality clones of an original hypha are distributed. Conidia can develop independently from the karyotic stage of the hypha that is anamorphs can occur both on haploid and dikaryotic mycelium.

2.2.3 Sexual Reproduction

A specific feature of the sexual reproduction of Ascomycetes and Basidiomycetes is that plasmogamy of haploid cells and karyogamy of two nuclei (n) to form a diploid nucleus (2n) are separated from each other temporally as well spatially by the dikaryophase (two-nuclei phase, dikaryon, n + n, ===) (Fig. 2.11). A dikaryotic hypha is one with two nuclei that derive from two haploid hyphae, but in which the nuclei are not yet fused by karyogamy.

Particularly in Basidiomycetes, the dikaryotic phase is considerably extended. By conjugated division of the two nuclei (conjugated mitosis), by division of the dikaryotic hypha, and by means of a special nucleus migration connected with clamp formation both daughter cells become again dikaryotic.

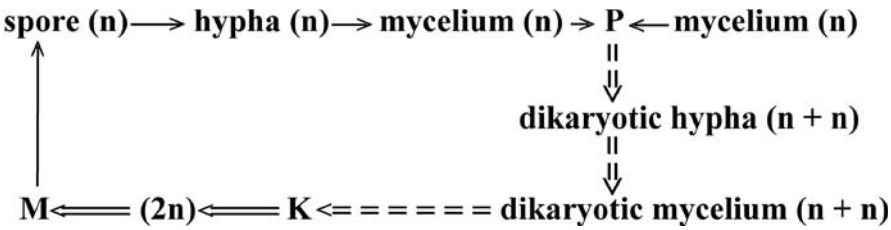

Fig. 2.11. Generalized scheme of nuclear condition of haplo-dikaryotic Ascomycetes and Basidiomycetes. → haploid (n), ===> dikaryotic (n + n), => diploid (2n), *P* plasmogamy, *K* karyogamy, *M* meiosis

2.2.3.1 Ascomycetes

The life cycle of a typical ascomycete is shown in Fig. 2.12 (also Müller and Loeffler 1992; Eaton and Hale 1993; Schwantes 1996; Jennings and Lysek 1999).

Haploid (n) spores (A, ascospores or conidia from an anamorph) germinate to haploid hyphae and after mitoses to haploid mycelium (B), which is

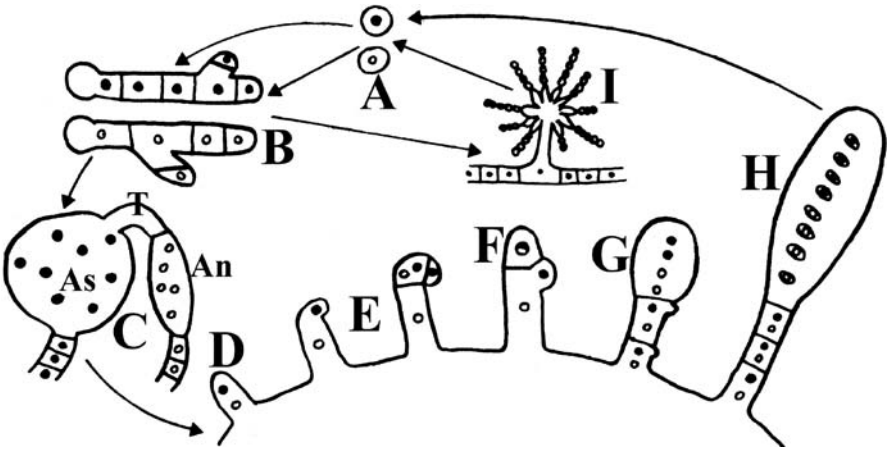

Fig. 2.12. Generalized life cycle of an euascomycete. *A* ascospores or conidia, *B* germinated monokaryons, *C* plasmogamy of ascogonium (*As*)-trichogyne (*T*) and antheridium (*An*), *D–G* section of ascogonium after incorporation of "male" nuclei, *D* ascogenous hypha, *E* hook formation, *F* karyogamy in the tip hypha, *G* dikaryon and ascus after meiosis, *H* ascus after mitosis with eight ascospores, *I* anamorph with conidia

the essential ascomycete with nutrition function and theoretically unlimited growth. Conidia may develop at the haploid mycelium as anamorph (I).

Within the fruit body, hyphae develop to gametangia ("sexual organs", C) connected with mitosis. The trichogyne (T, "copulation hypha"), which derives from the ascogonium (As, "female gametangium"), fuses (plasmogamy, gametangiogamy) with the antheridium (An, "male gametangium"). The nuclei from the antheridium migrate (therefore: male) through the trichogyne into the ascogonium. There may be various modifications of the generalized scheme: Antheridia are absent, and mono-nuclear spermatia (from an anamorph) fuse with the trichogyne (deuterogamy). Somatogamy of "normal" hyphae takes place (see Chap. 2.2.3.2). One sex is missing or not functional, and fertilization occurs between two nuclei of the same sex (automixis).

In the hymenial Ascomycetes (Ascohymeniales, wood-inhabiting Ascomycetes), the fruit bodies (ascocarps, ascomata) develop after the fertilization of the ascogonium from basal cells of the gametangia, and thus the fruit bodies predominantly consist of haploid hyphae (Fig. 2.13).

From the "pollinated" ascogonium, ascogenous hyphae develop, into which migrates each one pair of two genetically different (compatible) nuclei. In Ascomycetes, the dikaryotic phase is limited and without nutrition function. By means of hook formation (Fig. 2.12E) the short-lived hook mycelium and the ascus (meiosporangium) develop, in which karyogamy and meiosis occur. Before ascospore formation, there is commonly an additional mitosis, which brings the number of ascospores (meiospores) in the ascus to eight. The mature

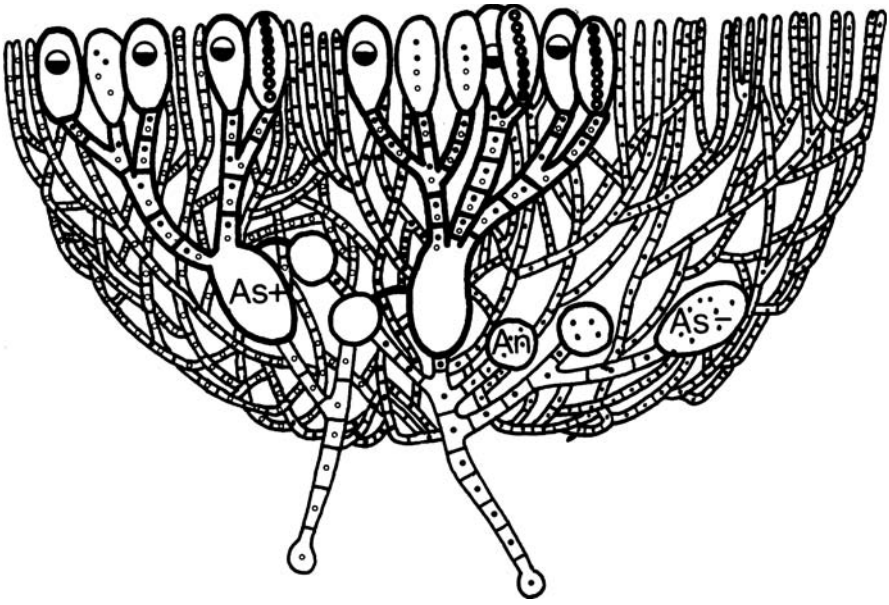

Fig. 2.13. Structure of a fruit body (apothecium) of an ascomycete predominantly consisting of haploid hyphae (*thin lines*, one nucleus), some dikaryotic hyphae (*thick lines*, two nuclei) and differently matured asci within the hymenium. *As-, An* ascogonium and antheridium before gametangiogamy, *As+* fertilized ascogonium

ascus is usually tube-shaped ("tube fungi"). The non-flagellate ascospores disper after disintegration of the ascus or via different opening mechanisms. The ascospores are mono-nuclear or after further mitosis multi-nuclear. They can be septate and show similar conidia characteristics of size, shape, color and wall sculpturing.

The relatively small fruit bodies (less than 1 mm in diameter) of the wood-inhabiting Ascohymeniales are the spherically closed cleistothecium, the pear-

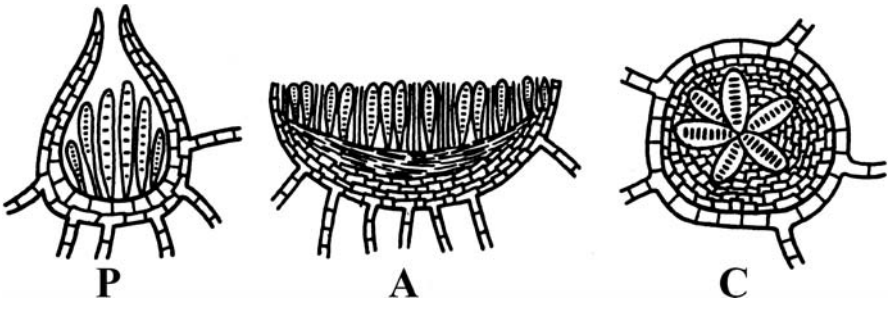

Fig. 2.14. Fruit body types of Ascomycetes. *P* perithecium, *A* apothecium, *C* cleistothecium

shaped perithecium, e.g., in several blue-stain fungi, or the disk-shaped apothecium (Fig. 2.14).

2.2.3.2 Basidiomycetes

The life cycle of a typical basidiomycete is schematically represented in Fig. 2.15.

The haploid basidiospore or conidium (A) germinates to the n-mycelium (B, monokaryon, primary mycelium). There are also asexual anamorphs in Basidiomycetes. According to Müller and Loeffler (1992), asexual anamorphs are supposed to occur almost just as frequently as in Ascomycetes: "they are named however only rarely with an own name, therefore hardly considered in the system of the Deuteromycetes and would be more frequent in the dikaryotic phase". A known example among the wood-decay Basidiomycetes is *Heterobasidion annosum* with its anamorph *Spiniger meineckellus*.

In the laboratory, monokaryons are capable of indefinite growth if they are regularly subcultured on fresh medium. In nature, characteristically the dikaryon or secondary mycelium develops. Basidiomycetes do not form sexual organs for plasmogamy, but monokaryotic hyphae come into contact one with another and fuse by somatogamy (C). If the nuclei are compatible, the dikaryon develops (D). This long-lived mycelium (Schwantes 1996) represents the essential basidiomycete that penetrates the substratum and absorbs nourishment, in the case of wood fungi with wood-decay function (D–G). In about half of the Basidiomycetes, the dikaryon grows by clamp connections (clamp mycelium):

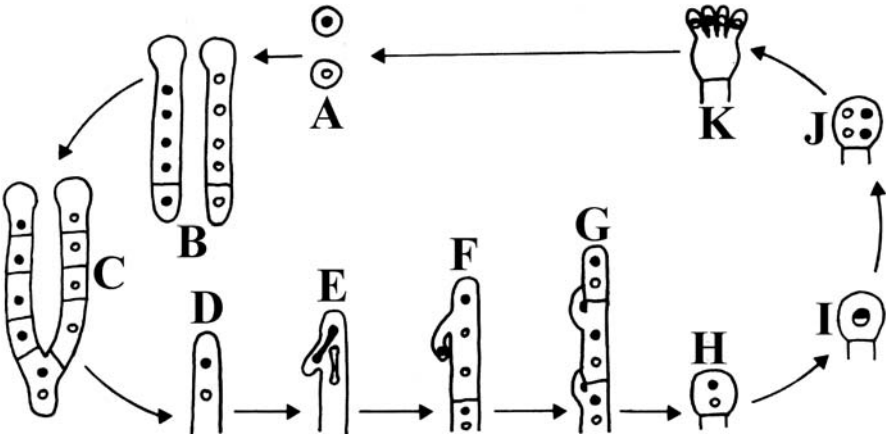

Fig. 2.15. Generalized life cycle of a homobasidiomycete. *A* basidiospores or conidia, *B* monokaryons after germination, *C* somatogamy, *D* dikaryon, *E–G* clamp formation, *H–K* basidium development, *I* karyogamy, *J* meiosis, *K* basidium with four basidiospores located in sterigmata

A short branch arises on the side of the apical hypha and bends over. After synchronous ("conjugate") division of the two nuclei (E), two daughter nuclei remain in the apical cell, one nucleus migrates into the branch (F), the branch end fuses with the subapical cell, and by septum formation, two dikaryotic hyphae have developed (G). Repeated conjugate divisions accompanied by septum formation result in an extensive dikaryotic mycelium (Jennings and Lysek 1999). Sometimes there are double or multiple (whorl) clamps (maximally eight) around one septum, e.g., in *Coniophora puteana* (four clamps). In a second method of dikaryotization, there is a division of the nuclei in the binucleate hypha followed by a migration of the daughter nuclei into the primary mycelium of the opposite mating type. The foreign nucleus in each mycelium divides and its progeny migrate from hypha to hypha through the septal pores until both parent mycelia have been dikaryotized (Alexopolus and Mims 1979).

Depending on external factors, like season (temperature, air humidity), nutrients and light, large fruit bodies (tertiary mycelium, basidiocarp, basidioma) develop on the secondary mycelium (Fig. 2.16).

In the fruit body of the hymenomycetes, the hymenium (fertile layer) develops (Fig. 2.16), in which the formation of basidia occurs (Fig. 2.15H–K). For

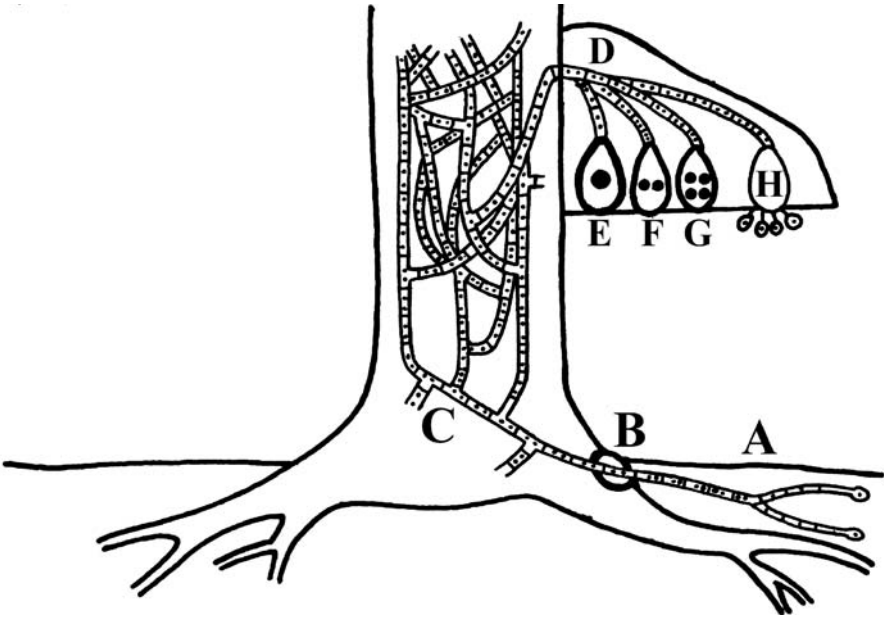

Fig. 2.16. Life cycle of a wood-decay basidiomycete. *A* haploid spores, hyphae, somatogamy and dikaryotic growth in the soil, *B* infection of the tree through a wound, *C* tree deterioration by the dikaryon, *D* fruit body formation (bracket); in the hymenium: *E* karyogamy, *F*, *G* meiosis, *H* mature basidium with four basidiospores