Fungal Degradation and Discolouration of Scots Pine

- a molecular approach

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Abstract

The aim of the present study was to use PCR-based methods to identify wood degrading and discolouring fungi in above ground conditions. The PCR-based Terminal Restriction Fragment Length Polymorphism (T-RFLP), cloning and sequencing procedures have been used to identify fungi. These results have been correlated and compared with microscope observations of fungi and decay types, mass loss, strength tests, decay intensity and environmental factors such as precipitation, temperature and altitude. An overview of current testing and evaluation of wood durability in above ground conditions has also been presented.

The results show that it is possible to identify wood degrading and discolouring fungi isolated directly from wood by using the T-RFLP technique. T-RFLP allows identification of more than one fungus in a sample in one single analysis according to their T-RFLP profile. In the two field test exposures used in this thesis, soft rot was the most abundant rot type found. The degree of soft rot correlated well with mass loss which was a better indicator for early stages of decay than impact bending, MOR and MOE.

Results do not provide any straightforward correlation between identified fungi, decay intensity, strength loss and mass loss or environmental factors. In this work accelerated tests, staple bed and double layer, were used as an attempt to speed up the decay progress. The results from these tests do not indicate any increased rate in wood deterioration. Staple beds exposed in the test field at the Swedish University of Agricultural Sciences in Ultuna were compared to the European standard field test, ENV 12037 exposed at the same time in the same field. After 2,5 years both test exposures were in the early stage of fungal colonisation indicating that the height above ground in the Ultuna field is of no importance for the initiation of wood deterioration by fungi.

Keywords: above ground, decay testing, fungi, natural durability, PCR, *Pinus sylvestris*, T-RFLP

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Appendix:

Papers I-IV

This thesis is based on the following papers, which are referred to by their Roman numerals:

I. Råberg, U., Edlund, M-L., Terziev, N., and Land, C. J. 2005. Testing and evaluation of natural durability of wood in above ground conditions – an overview. *Journal of Wood Science* 51, 429-440.

II. Råberg, U., Terziev, N., and Land, C. J. Soft rot colonization of Scots pine *Pinus sylvstris* (L.) sapwood in an accelerated above ground test. *Submitted to Journal of Wood Science.*

III. Råberg, U., Högberg, N. O. S., and Land, C. J. 2005. Detection and species discrimination using rDNA T-RFLP for identification of wood decay fungi. *Holzforschung* 59, 696-702.

IV. Råberg, U., Brischke, C., Rapp, A.O., Högberg, N. O. S., and Land, C. J. 2007. External and internal fungal flora of pine sapwood (*Pinus sylvestris L.*) specimens in above-ground field tests at six different sites in south-west Germany. *Holzforschung 61* (in press).

Introduction

Background

Currently there is a lack of accurate rapid and non-destructive methods for detecting and quantifying decay of wood in-service and there is a need to develop accelerated test methods for evaluating the efficiency of wood preservatives. To achieve this goal it will be necessary to develop improved methods for both detecting and quantifying the extent of wood decay. It is also important to develop a better understanding of the many variables that influence microbial decay rates and more knowledge on the process where various types of microorganisms are involved is needed. Once a better understanding of these relationships exists, it will be possible to develop improved wood decay test methods. It will however be important to introduce new methods in parallel with known tests and methods in order to allow acceptable comparisons. One question is what kind of techniques would be useful in wood science. The focus of this thesis concerns the fungal degradation of wood and the techniques that would be useful for detecting wood degrading and discolouring fungi in above ground conditions.

Wood durability

Several biological organisms decompose wood if the correct environmental conditions are available. Fungi, bacteria and insects may attack wood and cause significant damage. The natural recycling process of organic material is an important process in nature, but becomes problematic when wood is degraded while in-service. However, wood is a popular construction material because of its inherent good qualities like strength in relation to weight. Furthermore it is a renewable resource and considered environmentally friendly.

The durability of wood determines the service life of wooden constructions and is a result of the materials resistance to wood-destroying organisms under actual environmental conditions. In order to predict the service life of wooden constructions, it is important to consider environmental factors such as moisture content, wood temperature, accessible nutrients and the types of wood-decaying fungi present (Brischke, Bayerbach & Rapp, 2006).

Two standards in Europe, ENV 12037 (1996) and EN 330 (1993) describe how to evaluate wood used in above ground conditions (use class 3[*]). The general

[*] Use class (UC). The European standard, EN 335, defines use classes in order to give users information and guidance to determine the appropriate use class and to select a suitable level of durability (natural or preservative treated) against biological agencies. UC 1-above ground, covered, no wetting is expected; UC 2-above ground, covered, occasional wetting is expected; UC 3- above ground, not covered, frequent wetting is expected; UC 4-in contact with ground or fresh water, permanently wetting is expected; UC 5-in contact salt water, permanently wetting is expected.

concept is that the service life of a wood species and wood-modification are measured and compared to a defined reference. These references are *Pinus sylvestris* (L.) sapwood and CCA (chrome-copper-arsenic) impregnated *Pinus sylvestris* (L.) sapwood with a retention of 5,3 kg/m^3. The evaluation is subjective and considers discolouration and decay using the pick test (see below). The duration of this test is for a minimum of five years.

Structure of Scots pine

In this work, the softwood species Scots pine (*Pinus sylvestris* (L.)) was used as a test material. The cross section of the trunk of Scots pine (Figure 1) may distinguish between the brighter sapwood and the darker heartwood. The sapwood store and conduct nutrients, while in the heartwood these processes are discontinued. The extractives are mainly located in the heartwood and give the wood a darker appearance.

Growth of wood in temperate regions results in formation of growth rings, so called annual rings (Figure 1). Annual rings are formed as a consequence of one growth period per year. The annual rings can easily be seen because of differences in cell wall thickness. The cells formed in the spring (e.g. earlywood) are thin and have a lighter appearance than the thick-walled cells formed during the summer and early autumn that forms the latewood.

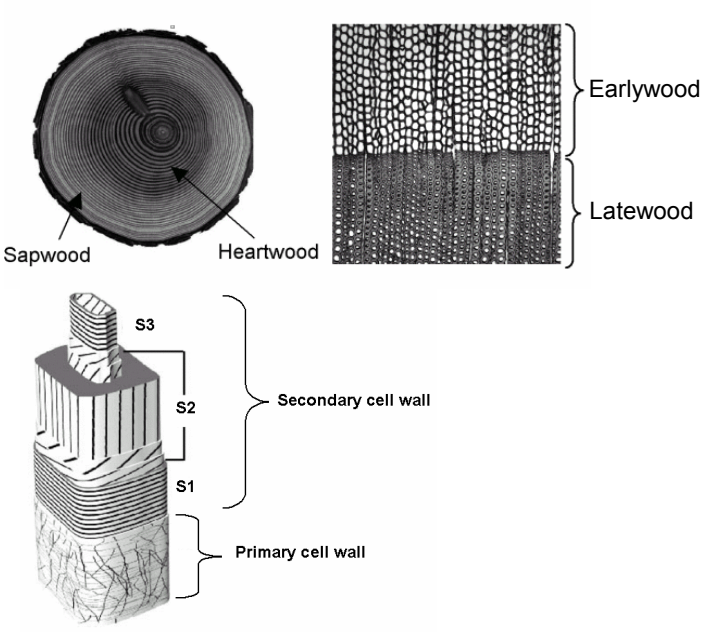

Figure 1. Structure of Scots pine showing sapwood and heartwood, annual rings and the different layers forming the cell wall. (Image of cell wall layers is from Brändström 2002).

While the mechanical strength of softwood species such as Scots pine comes from the elongated and thick latewood tracheids, the earlywood has a water and nutrient transfer function. Cells surrounding resin canals are called parenchyma cells. Their function is the storage of nutrients (Eaton & Hale, 1992; Dinwoodie, 2000). Wood cells have two cell walls, the primary and secondary cell walls (Figure 1) and individual cells are bound together by the middle lamella (Figure 1). The primary cell wall has randomly oriented cellulose microfibrils and is rich in pectin and glycoproteins. The secondary cell wall consists of three layers referred to as S1, S2 and S3. These layers differ in microfibril orientation and lignin content. In general, softwood consists of 40-45 % cellulose, 25-30 % hemicellulose, 15-35 % lignin and 1-4 % extractives (Eaton & Hale, 1992; Dinwoodie, 2000).

The wood structure provides the substrate for fungal growth and development. During the decay process fungal hyphae may grow into the wood via the rays and then spread further. Depending on the decay type the fungal hyphae may be localized in the cell lumen or actually within the secondary cell walls.

Wood decay types

A rot fungus may belong to one of three major groups causing different types of decay known as: 1) soft rot, 2) brown rot, or 3) white rot. This artificial grouping primarily reflects the nature (e.g. morphological appearance) of degradation of the wood. The soft rot fungi belong to the ascomycetes and Deuteromycetes (Fungi Imperfecti), and can degrade cellulose in the wood cell wall S2 and S1 layers by creating diamond-shape cavities. This leads ultimately to a softening of the wood. A common soft rot fungus is *Chaetomium globosum* and many important soft rot fungi belong to the genus *Phialophora* (Nilsson & Henningsson, 1982; Blanchette et al., 1990; Daniel & Nilsson, 1998). Brown rot fungi belong to the basidiomycetes. They degrade primarily the cellulose and hemicelluloses and modify the lignin. This causes a brownish colouring of the wood and ultimately the wood cracks into cubic forms and crumbles when dry. White rot fungi belong to the basidiomycetes and higher ascomycetes and degrade the cellulose, hemi-celluloses and lignin (Nilsson et al., 1989; Daniel, 2003). Initially the wood becomes darkened and can be mistaken for brown rot; later the wood becomes brighter than normal wood and may form a fibrous mass. The rot fungi are the major wood degraders and can cause severe strength loss of the wood, while staining fungi (i.e. discolouring fungi see below) do not normally degrade wood nor cause significant strength loss. Nevertheless, the discolouring fungi are of great concern because of the discolouration they cause and loss to wood quality (Zabel, & Morell, 1992; Eaton & Hale, 1993; Dinwoodie, 2000).

Discolouring fungi

Moulds and staining (i.e. blue stain and sapstain) fungi are referred to as discolouring fungi and belong to the Ascomycetes and Deuteromycetes (Fungi

Imperfecti). Normally, moulds and blue stain fungi do not degrade lignified cell walls, but use the contents of ray parenchyma cells for growth into the wood, although there are a few blue stain fungi known to cause soft rot (for example *Alternaria spp.*, and *Phialophora spp*.) (Käärik, 1974). Moulds grow on the wood surface and their spores cause discolouration (black or green), the mycelium being colourless. Moulds may cause allergies and one of the most common allergens is *Alternaria spp.* common on pulpwood (Schmidt, 2006). *Aspergillus* is a typical wood mould that produces the toxic mycotoxin aflatoxin that can cause liver damage (Deacon, 2006).

Staining fungi have pigmented mycelia that penetrate the wood through the rays, bordered pits and cell lumen (Käärik, 1974). Staining fungi do not normally attack the wood cell wall cellulose and therefore they have no major affect on the strength properties. Stain fungi are spread by either insects (e.g. *Ophiostoma*) or by air. Common airborne staining fungi belong to *Aureobasidium pullulans, Hormonema dematioides*, *Rhinocladiella atrovirens*, *Phialophora spp*. *Cladosporium spp.* and *Phoma spp.* (Deacon, 2006; Schmidt, 2006).

Ways of grading wood decay

Currently the most common ways to evaluate durability or degradation of wood are by visual and microscopic evaluations, the pick test, and tests by means of density-mass- or strength loss. These approaches are described in more detailed in paper I. The methods used in this thesis are briefly described below.

Mass loss

Mass loss is commonly used in laboratories to assess the degradation of wood. Reasons for this are the availability of balances in the laboratory and that the variation between samples is low. Wood blocks are conditioned by oven drying (at 103°C) or at constant temperature and relative humidity (for example 20°C, 65% RH) and their weights are measured before and after testing. Mass loss is expressed as a percentage of the original dry weight (Eaton & Hale, 1993). Earlier, mass loss was considered as the most reliable way to compare results from different experiments involving wood decay. The main drawback of mass loss is its inability to detect the early stages of decay. Strength toughness and impact bending strength (see below) are the most sensitive measures for early stages of decay (Wilcox, 1978).

Pick test

The pick test is a simple method for detecting surface decay in poles and timber. In practice, a knife is driven into the wood at an acute angle and bent back in order to snap a small piece of wood from the surface. The break characteristics of the splinter removed are then examined. A brash break reflects reduced strength and the possible presence of decay, whereas a splintery break reflects sound wood. The pick test measures toughness and is fairly sensitive to early decay (Wilcox, 1983; Morell et al., 1986). The drawbacks of the pick test is the relatively large sample

removed and the inability to accurately assess the internal conditions of the wood (Zabel & Morell, 1992). Decay may be detected as early as with 5-10 % mass loss by the pick test. This is close to the level when decay first becomes detectable under the microscope. Considerable wood strength is lost in the early stages of decay with certain fungi (e.g. brown rot fungi) and therefore a high sensitivity of the test is desirable. The pick test is a subjective test where accuracy and reproducibility may vary with factors like experience of the performer, latewood content and fibre orientation (Wilcox, 1983).

Strength tests

Strength tests involve irreversible destructive testing of specimens to failure. Even with a uniform set of specimens, considerable variation in results are obtained. The strength of wood is defined as its ability to resist loads to the point of ultimate failure. A variety of other mechanical properties are also important such as characteristics of stiffness and creep. Strength test results are usually expressed as the energy applied per unit area or volume. There are many factors, that need to be considered when strength is assessed including density, grain angle, uniformity (e.g. clear specimens or specimens with defects, particularly knots and splits), moisture content, temperature, rate of loading, age of wood and previous histories of load. All these factors interact and should be considered in strength evaluation (Zabel & Morell, 1992; Eaton & Hale, 1993; Dinwoodie, 2000). In this work, standard procedures for impact bending and static three-point bending have been used (Paper II).

Impact bending

The decrease of toughness or resistance to impact loading caused by fungi is the most sensitive property to detect early stages of decay, followed by static bending properties (Wilcox, 1978). In this test, the test piece is supported over a span and a mass is dropped onto the test piece from a defined height (SKANORM 13, 1992; Dinwoodie, 2000).

Bending

Wood modulus of elasticity (MOE) decreases during decay. MOE is determined by using a three-point bending machine. This is a sensitive strength property, where strength losses of 50-70 % can be expected at a mass loss of 5-10 % (Wilcox, 1978). Many studies have confirmed that strength loss is more sensitive than mass loss during early stages of decay (Reinprecht & Tiralová, 2001; Curling, Clausen & Winandy, 2002). Here the test piece is supported over a span carried on roller bearings, the load is applied to the centre of the test piece at a constant rate. The orientation of the annual rings is parallel to the direction of loading. Three strength properties are usually determined from the bending test, 1) Modulus of rupture (MOR); 2) Work to maximum load; and 3) Total work (SKANORM 7 & 8, 1992; Dinwoodie, 2000).

Fungal succession in softwood

The fungal succession of construction wood has a somewhat different range of species compared to living trees or green wood, but follows the same general pattern of microbial succession with bacteria, stain- and mould, soft rot and brown or white rot fungi (Käärik, 1974; Eaton & Hale, 1992; Dix & Webster, 1995; Deacon, 2006; Schmidt, 2006). This sequence of events may however change when using treated wood.

It is generally assumed that fungal spores are the principal mode of infection for wood exposed above ground under conditions suitable for decay. For fungal spores to germinate, they only need water since nutrients are stored in the spore. To continue to grow, fungi need favourable conditions including sufficient nutrients, correct microclimate, moisture and absence of competitors. Normally these conditions do not exist at the same time, therefore the presence of spores does not ensure that the wood decay process will begin. Fungi can also produce different kinds of spores, such as basidiospores, chlamydospores and conidia. These spores may have different requirements for germination (Nicholas & Crawford, 2003; Deacon, 2006).

The leaf inhabiting fungi *Epicoccum nigrum* and *A. pullulans* are frequently isolated from woody stems but soon disappear as the decay communities develop. Other common endophytes on *Pinus* species are *Cryptosporiopsis*, *Daldina* (anamorph), *Fusarium lateritum*, *Hormonema*, *Hypoxylon* (anamorph), *Nodulisporium*, *Phoma*, *Phomopsis* (Diaporthe) and *Xylaria* (anamorph) (Dix & Webster, 1995).

The moulds are closely followed by the soft rot fungi. In this group *Phialophora* species are common in standing trees and stumps while *Chaetomium*, *Humicola* and *Doratomyces* are more common in construction wood (Dix & Webster, 1995). Moulds have a potential role in wood decay as modifiers of wood and together with soft rot fungi may help to prepare wood for invasion by basidiomycetes. They may disrupt pit membranes and thus assist the passage of hyphae through the tissues. Some soft rot fungi can also be involved in the decay of toxic phenols (Dix & Webster, 1995). It is important to note that colonisation of moulds and soft rot is not essential for the establishment of all basidiomycete decay fungi on wood (Rayner & Boddy, 1988). Mixed fungal communities decay wood quite rapidly and the micro-fungi of wood are important in this respect (Dix & Webster, 1995). Moulds in particular have an important role to play. They can enter a mutualistic association with wood-decay basidiomycetes and accelerate the utilisation of lignin and cellulose. *Leptodontium* and *Rhinocladiella* have an interesting ecology and occur deep in decaying wood in narrow bands of undecayed tissues between the zone line that demarks the boundaries of the basidiomycetes mycelia (Dix & Webster, 1995).

Wood decay of poles and boards are mainly caused by *Coniophora puteana, Fomitopsis pinicola, Gloeophyllym trabeum, Paxillus panuoides, Phlebiopsis gigantea, Sterum sanguinolentum* and *Trichaptum abietinum*. On wood chips,

Bjerkandera adusta, *Gloeophylllum spp*., *Phanerochaete chrysosporium* and *Trametes versicolor*. On structural timber used outdoors *Antrodia vaillantii, Heterobasidium annosum, Lentinus lepideus, Leucogyrophana pinastri, Postia placenta, Phanerochaete sordaria, Phlebiopsis gigantea, Serpula himantioides, Sistotrema brinkmanni, Trametes versicolor* and *Trichaptum abietinum* are important wood degrading fungi (Clubbe, 1980; Richardson, 1993; Schmidt, 2006).

Molecular methods for identification of fungi

The Deuteromycetes are identified and described depending on their morphology, colour and development of conidia and conidiogeonus cells. Ascomycetes and basidiomycetes are identified based on their fruit body macro- and microscopic characteristics using keys and illustrations (Huckfeldt & Schmidt, 2006; Schmidt, 2006). For basidiomycetes that have only established mycelium, keys are based on microscopic characteristics of the hyphae, growth parameters and other characteristics (Rayner & Boddy, 1988; Schmidt, 2006). Molecular methods to characterise, identify and classify fungi do not depend on subjective judgement that may occur using traditional methods, but are based on objective information on the DNA from the studied fungi. Some genera among the rot fungi, such as *Antrodia*, *Coniophora* and *Leucogyrophana,* are virtually impossible to discriminate using traditional methods of identification (Schmidt, 2006).

Molecular targets in this thesis

Before molecular assays, the target DNA needs to be selected. One popular DNA region for identification of fungal species is nuclear ribosomal DNA (nrDNA). nrDNAs are present in all organisms at high copy numbers and are assumed to have little sequence divergence between rDNA copies within a single individual and divergence between species (Diehl, McElroy & Prewitt. 2004; Schmidt, 2006). The fungal nrDNA unit consists of three genes, the large subunit (LSU) rRNA (28 S) gene, the small subunit (SSU) rRNA gene (18S), and the 5.8 S rRNA gene, separated by internal transcribe spacer (ITS) regions (Figure 2). The SSU is highly conserved and contains low levels of sequence variation. LSU is more variable and is useful to differentiate genera and species of fungi. The conserved regions are used for phylogenetic analysis of genera, families and orders. Based on the conserved regions, universal and specific primers can be developed to detect large number of fungal species using the ITS 1 and ITS 2 regions (White et al., 1990; Mitchell & Zuccaro, 2006).

After amplification by the Polymerase Chain Reaction (PCR), the amplicons are most often used in further analysis of which some are described below.

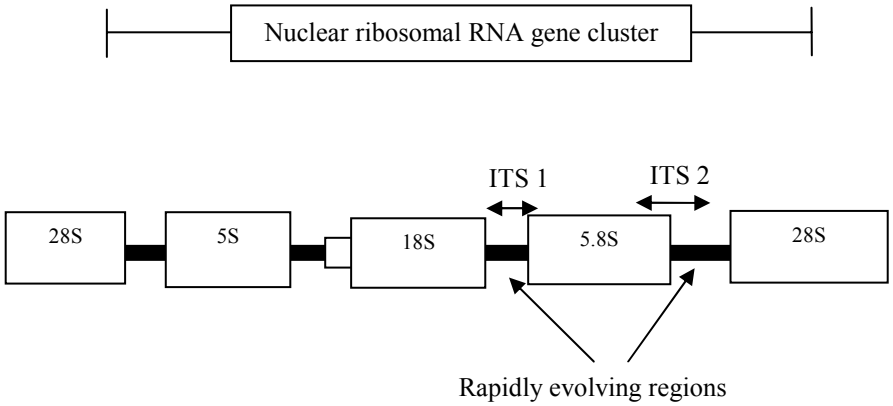

Figure 2. Showing the nuclear ribosomal DNA gene cluster that is repeated over the fungal genome. Different molecular targets are indicated. (based on Michell & Zuccaro, 2006; Schmidt, 2006).

Community studies

As the limitations of culturing methods became clear many different techniques for evaluating microbial communities were developed. The majority of these techniques use the Polymerase Chain Reaction (PCR) to amplify genes of interest directly from environmental samples without culture bias. Such PCR-based methods include Random Amplification of Polymorphic* DNA (RAPD), Amplified Ribosomal DNA Restriction Analysis (ARDRA), Species Specific Priming PCR analysis (SSPP), Thermal and Denaturing Gradient Gel Electrophoresis (TGGE and DGGE) and Terminal Restriction Fragment Length Polymorphism (T-RFLP) patterns or profiles. All of these methods produce a pattern or profiles of nucleic acids amplified from a sample that reflects the microbial community structure (Figure 3) (Jellison, Jasalavisch & Ostrofsky, 2000; Kitts, 2001; Schmidt, 2006; Mitchell & Zuccaro, 2006).

RAPD have been used to show polymorphism within species (Theodore et al., 1995; Schmidt & Moreth, 1998; Göller & Rudolph, 2003). RAPD does not require previous information of the target DNA, but requires pure cultures or clones. At least four primers should be used to obtain reliable results (Schmidt, 2006). This method cannot be used to identify fungi from unknown samples.

ANDRA is a Restriction Fragment Length Polymorphism (RFLP) based on rDNA. Before analysis, the ITS region is amplified by using universal primers (e.g. ITS 1 and ITS 4 (White et al. 1990)) and primers anneals at the conserved region of the rDNA, allowing ITS amplification of fungi without prior knowledge of their

* Polymorphism means many forms and describes that individuals in a population exist in two or more clearly distinct forms (i.e. morphs) with different appearance and physiological properties. Polymorphism also reflects variations in an organisms DNA sequence that may or may not be expressed in its phenotype.

ITS sequence. One or more restriction enzymes then digest the amplicot with specific recognition sites on the DNA. This method has been used to discriminate between isolates of wood decay fungi (Schmidt & Moreth, 1999; Jasalavich, Ostrofsky & Jellison, 2000; Horisawa et al., 2004; Diehl, McElroy & Prewitt, 2004). The advantage with this method is that it is fast and inexpensive, however one important limitation is the difficulties for identification by fragment length comparison between different sample sets with high accuracy.

Seven indoor rot fungi have been studied using SSPP as a tool for detection of these fungi in a non-cultured or cloned procedure (Moreth & Schmidt, 2000; Schmidt, 2000). The method is fast and precise and already used commercially in Germany (Schmidt, 2006). The limitation with this method is that it is not suitable for fungi in closely related genera that have rather similar ITS sequences and also for those fungi with intra-specific variation (Schmidt, 2006).

DGGE analysis has been used by Vainio and Hantula (2000) on fungal samples of spruce and by Ray and Dickinson (2006) for detection and discrimination of *A. pullulans* and *H. dematioides* in various field samples.

T-RFLP is like ANDRA, based on profiles that are generated and analysed in a series of steps that combine PCR, restriction enzyme digestion and gel electrophoresis. DNA extracted from a sample is subjected to PCR using primers homologous to conserved regions in a target gene. In T-RFLP, one or both primers are labelled on the 5'-end, usually with a fluorescent molecule. The amplified DNA fragments (amplicons) are then digested with a restriction enzyme. The digested amplicons are subjected to electrophoresis in either a polyacrylamide gel or a capillary gel electrophoresis apparatus - usually a DNA sequencer with a fluorescence detector - so that only the fluorescently labelled terminal restriction fragments (TRFs) are visualised. Most studies use an automated fragment analysis program that calculates TRF length (bp) by comparing TRF peak retention time to a DNA size standard. These programs integrate the electropherrograms and return TRF peak height and area. The patterns of TRF peaks can then be numerically compared between samples using a variety of multivariate statistical methods. In addition, individual TRF peaks in a pattern can be identified by comparison to a clone library or by predictions from an existing database of sequences (Marsh, 1999; Kitts, 2001).

Several reports using T-RFLP analysis have been published, mainly on bacteria (Clement et al., 1998; Marsh, 1999; Kitts, 2001; Blackwood et al., 2003), but also a few on fungal communities (Marsh, 1999; Lord et al., 2002; Dickie, Xu & Koide, 2002; Råberg et al., 2006, 2007).

All these methods produce a pattern or profile that corresponds to the fungal community structure. The T-RFLP approach gives robust and reproducible data, which allows a rapid analysis of large amounts of data. Software is available that automatically digitalised the fragment data, while other methods need to be digitalizes manually. This means that the T-RFLP profile gives an output with higher precision, which can be used to search for individual taxa within a

community profile. Profiles generated by DGGE are best compared on the same gel because of the lower resolution inherent to the electrophoresis system (Kitts, 2001). The advantage of DGGE over T-RFLP is the possibility to reuse the DNA of a certain profile for identification of a direct cloning of the profile elements, whereas the T-RFLP profile is destructively sampled and the DNA cannot be reused. This means that a database of T-RFLP profiles is necessary for identification of a specific profile in a T-RFLP analysis. After establishing such a library, a search for a specific fungal species or community structures will be facilitated.

The ITS sequence for a number of wood decay fungi have been sequenced (Schmidt & Moreth, 2002; Schmidt & Moreth, 2003; Jellison, Jasalavich & Ostrofsky, 2003; Högberg & Land, 2004; Råberg, Högberg & Land, 2005) and deposited in international databases free to use (The European EMBL, http://www.ebi.ac.uk/embl; The American NCBI GenBank, http://www.ncbi.nlm.gov/genbank; The Japanese DDBJ, http://www.ddbj.nig.ac.jp). Sequences of the ITS region can be used to identify unidentified fungal samples through sequence comparison by Basic Local Alignment Tool (BLAST) (Altschul et al., 1997). DNA sequencing requires amplicons from pure cultures or clones and gives comprehensive information. Inexpensive sequencing services have become more and more common and have made this method readily available for smaller laboratories.

In this thesis T-RFLP and sequencing have been used to identify fungal species.

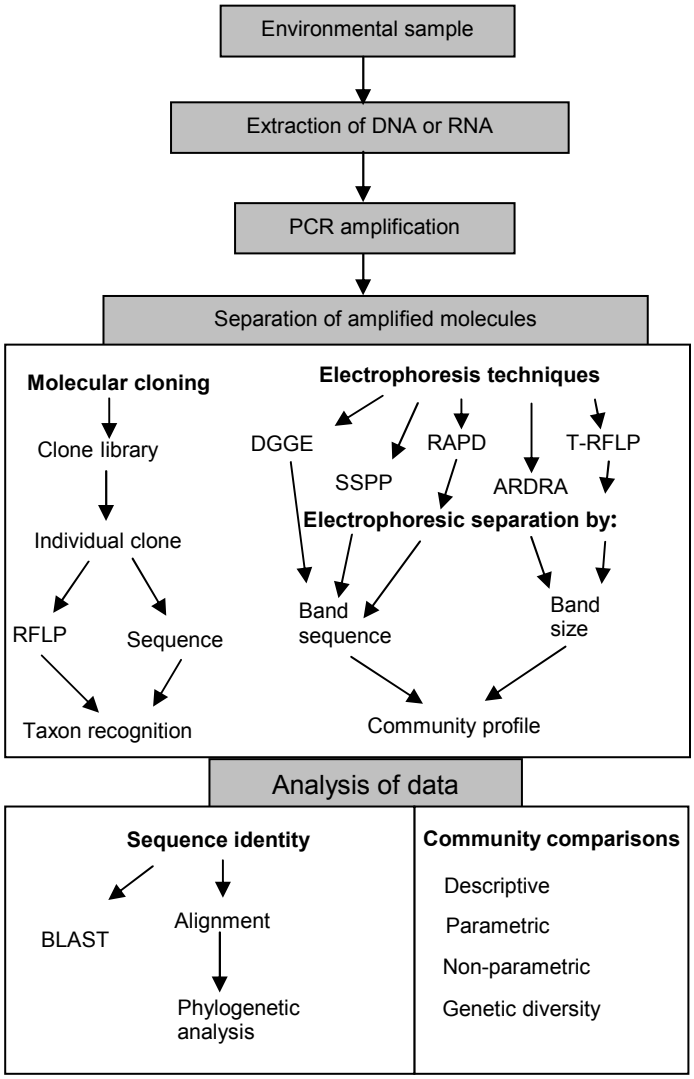

Figure 3. An overview of different methods for community studies (based on Mitchell & Zuccaro, 2006).

Objectives of the thesis

The overall aim of the work described in this thesis was to identify wood discolouring and degrading fungi using molecular techniques, and study the relation between fungi, environment and biological degradation.

More specifically the objectives were:

- Identify fungi degrading wood in above ground conditions, and correlate fungal diversity with decay intensity, types of rot present, and characteristics of the test sites such as precipitation, temperature and height above sea level (**paper IV)**;

- Contribute with information on fungi to the Department of Wood Science culture collection and construct a molecular database based on sequence and T-RFLP information of common rot fungi (**papers III and IV**);

- Combining traditional ways of testing and evaluate wood degradation in above ground conditions with molecular techniques (**papers II and III**);

- To review wood durability testing above ground and their significance for detecting and evaluating wood decay. Are new techniques and possibilities useful in the testing and evaluation of wood decay? (**paper I**); and,

- Correlate wood decay intensity with detected fungi (**paper IV**).

- Compare microscope and molecular early detection of wood decay fungi (**II** and **III**).

Materials and methods

Study site and sampling

In this thesis two field exposures have been used for the testing and evaluation of fungal flora and its effect on wood specimens. In this work the wood species *Pinus sylvestris* (L.) was used. A staple bed test (Figure 4) at Ultuna test field in Uppsala (Sweden) was used for colonisation of pine sapwood. The site and sampling used is described in paper II. Sites used in southwest Germany are described in paper IV. At the German sites, double layer tests (Figure 4) were used to determine the external and internal fungal flora of pine sapwood. The sampling procedure is described in paper V.

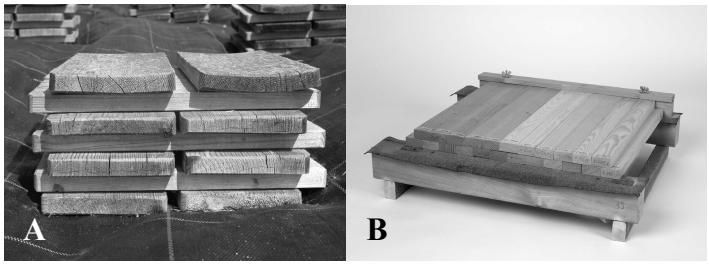

Figure 4. Staple bed (A) and double layer (B) test set-ups.

Microscopic evaluation of decay types

For the detection of the different decay types a light microscope with magnification between 10X – 63X was used. Presence and absence of white-, brown-, and soft rot, mycelia and visual stain were noted. The presence of soft rot was visualised in sections taken from the wood samples and graded between 0-4 according to Henningsson et al. (1975) (Figure 5). The grading follows: 0) no cavities; 1) few scattered cavities; 2) numerous cavities in most cells; 3) abundant cavities often fused together; and 4) almost total or complete disintegration of the secondary wall of cells. The evaluation method is described in detail in paper II.

Colonisation test

To establish how early it is possible to isolate and detect fungi in wood with PCR, a soil bed test was conducted. The test is described in detail in paper III, and basically consists of glass flasks filled with sterile soil, small pine sapwood specimens and one fungus in each flask. In this study the brown rot fungi *Poria placenta* and *Coniophora puteana* were used. The tests were run for 8-9 weeks and every week one of the glass flasks was examined for fungal colonisation of the wood. The results from PCR were compared with fungal hyphae detection using light microscopy (40X) (paper III).

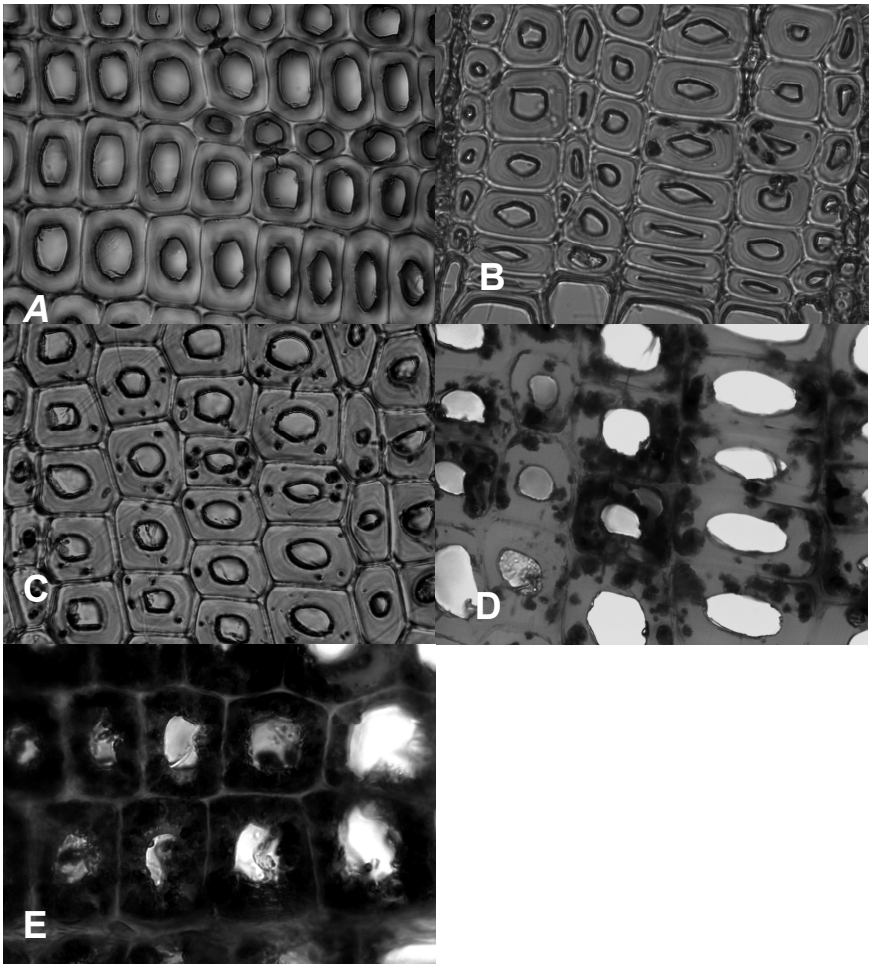

Figure 5. The light micrographs show degree of soft rot attack: A) 0 no cavities; B) 1 - few scattered cavities; C) 2 - numerous cavities in most cells; D) 3- cavities abound, often fused together; and E) 4 - almost total or complete disintegration of the secondary cell wall.

Strength tests

Wood specimens from the stable bed test were sampled for strength loss every sixth month. They were removed from the field and the modulus of elasticity (MOE) and impact bending strength were measured.

The modulus of elasticity (MOE) was determined by the 3-point bending test in a universal test machine (Alwetron TCT 50) according to SKANORM 8 (1992). Modulus of elasticity (MOE) was calculated according to the equation:

$$MOE_{(Mpa)} = \frac{\text{Load} \times \text{span}^2}{\text{Rate of bending} \times \text{width of the sample} \times \text{thickness of the sample}^3}$$

Impact bending was determined in a pendel machine with a metal hammer (150 J) machine (FRANK) according to SKANORM 13 (1992). Impact bending was calculated according the equation:

$$\text{Impact bending (kJ/m}^2\text{)} = \frac{1000 \times \text{rate of impact}}{\text{Width of the sample} \times \text{thickness of the sample}}$$

Mass loss and moisture content

Wood specimens in the staple bed test were sampled for mass loss every sixth month. Mass loss was measured during the test period by using the dry weight at the time of sampling compared with the dry weight of specimens at the start of the test period. The mass loss is expressed as a percentage of the original weight. Specimens were cleaned from any adhering mycelia before they were conditioned and weighed. The weight was measured at constant temperature and relative humidity (12 % RH, 20 °C). Mass losses were calculated according to:

$$\text{Mass loss (\%)} = \frac{m_o - m_d}{m_o} \times 100$$

where m_o is dry mass prior to test, and m_d is dry mass after the test.

The moisture content in the wood specimens were measured monthly (except December-March, when specimens were frozen) over the test periods. The moisture contents was calculated according to:

$$\text{Moisture content (\%)} = \frac{m_w}{m_o} \times 100$$

where m_w is wet mass and m_0 is initial dry weight.

Identification of fungi

The identification of fungi was reported in papers III, IV and V. The methods used were based on extraction, purification and amplification of DNA, followed by either cloning and sequencing or analysis of T-RFLP fragments. The protocol used was described in the papers outlined above. In brief, after DNA extraction the ITS region was amplified. The PCR product was then digested by restriction enzymes into fragments of different length and separated by capillary electrophoresis for fragment size determination. The PCR product may contain one or several fungi, which could be identified by their fragment lengths. To relate a specific fragment size to fungal species, selected PCR products were cloned, sequenced and digested by restriction enzymes for determination of the fragment size. This gave a fungus

specific T-RFLP profile for a fungus that could be used to identify a particular fungus or several fungi in an environmental sample (Figure 6).

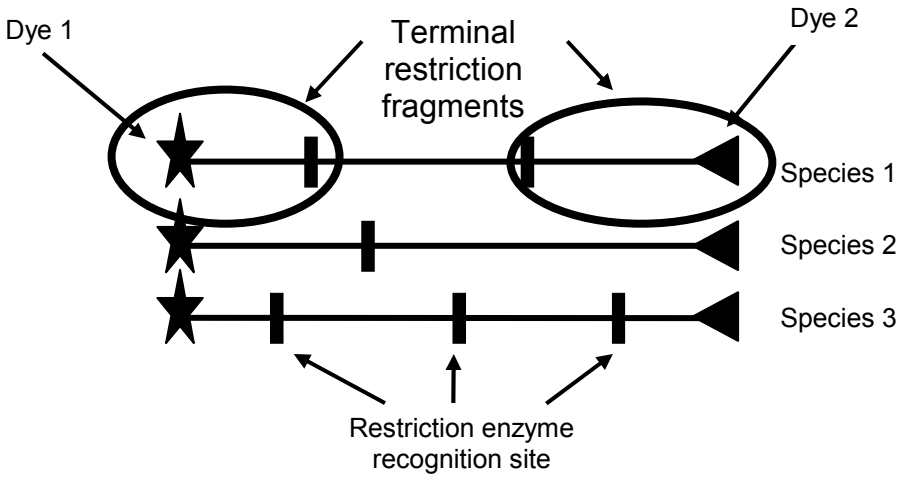

Figure 6. This shows PCR products of three fungal taxa using PCR primers coupled with a fluorescent dye (dye 1 and 2). The dye can be attached to one primer or as shown here to both primers. The PCR products are digested by a restriction enzyme that cuts the sequence at a specific location. Since the sequence varies between different species, the enzyme will cut at varying location on the PCR product creating fragments of the original PCR product. Only the ends of these fragments will have dye coupled and the other fragments will be without dye. When these fragments are analysed in an automated sequencing machine, only those fragments that carry a fluorescent dye will be read. This means that only the terminal fragments will be determined. Thus, this is called Terminal Restriction Fragment Length Polymorphism (T-RFLP).

Results

Overview

Two accelerated tests, staple bed and double layer were used in this thesis as an attempt to speed up the decay progress. Results do not indicate any faster progress in wood deterioration in these tests compared to the current European standard field test, ENV 12037 performed in Uppsala (paper II). After 2.5 years field exposure for the staple beds and 3-4 years for the double layer test, both tests showed an early stage of fungal colonisation and the course of decay was also in an initial stage (papers II and V).

Microscope evaluation every sixth month of the specimens in the staple bed exposed in the field revealed soft rot abundance (paper II). *Lecythophora hoffmanni* was isolated and identified as a soft rot fungus. A good correlation between the mass loss and degree of soft rot for both open field (r^2=0,70) and shaded exposures was recorded (r^2=0,68) (Table 1). Surprisingly, there was no significant correlation between the mass loss and strength loss of pine sapwood (Table 1).

Early stages of fungal colonisation can be detected by PCR. To show this, microscope detection of mycelia was compared with PCR detection of fungi (paper III). PCR detected the fungi isolated directly from the wood specimen two weeks after inoculation (Table 2). Results show that it is possible to identify wood degrading and discolouring fungi, isolated directly from the wood using the PCR-based T-RFLP technique. T-RFLP allows identification of more than one fungus in a sample in one analysis according to their T-RFLP profile (Figure 7).

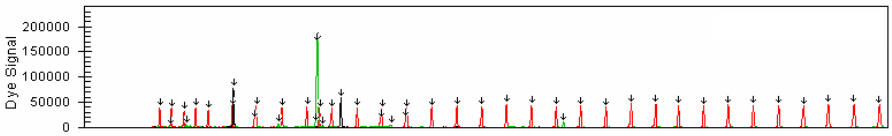

Figure 7. Ferrogram of a typical output from a single T-RFLP analysis. In some samples more than one fungus is present and therefore more peaks are detected. The height of the peak is further related to the amount of fungus present in the T-RFLP analysis.

In both field test exposures, soft rot was the most abundant type type. This is consistent with the general concept of the colonisation order of fungi in construction wood. *Phialophora spp*. appear to be common in terrestrial environments and have been frequently isolated in this work, while *Chaetomium globosum,* possibly the most commonly studied soft rot fungi species (Blanchette et al., 1990) and used in laboratory decay tests was not isolated during this work.

Paper I

Paper I gives an overview of present testing and evaluation of natural durability of wood in above ground conditions that are used in Europe. It presents the European standard tests that are used both in the laboratory and during field testing. The methods on how to evaluate the soundness of wood are also briefly described. A few examples of accelerated tests are also discussed. In general they consist of fungus cellar tests or field exposures focusing on trapping water at various heights above ground.

Laboratory and field tests have different advantages and drawbacks. Therefore, a combination of laboratory tests and field-testing is a good solution to obtain a first indication that should later be confirmed using field tests. In order to obtain more realistic laboratory testing, a thorough knowledge of the course of fungal colonisation is needed to be able to mimic the interactions in the laboratory.

Paper II

In this paper, an accelerated test was used as an attempt to speed up the progress of wood decay. The European standard test for above ground conditions (ENV 12037) recommends optimal conditions for fungal growth by placing one test set-up in a site shaded by trees. In this study at the Swedish University of Agricultural Sciences in Ultuna, the test exposure (open and shaded field conditions) did not affect fungal growth. There was no indication that mass loss and decay progress were favoured by shaded exposure compared with the open field. This was confirmed by comparing the degree of soft rot attack by means of light microscopy. The degree of soft rot followed that described by Henningsson et al. (1975). There was a good correlation between the mass loss and soft rot degree for both open field (r^2=0,70) and shaded exposures (r^2=0,68) (Table 1). Surprisingly there was no significant correlation between the mass loss and strength loss of pine sapwood (Table 1). Strength losses are considered to be the most sensitive way to detect early stages of decay (Wilcox, 1978; Reinprecht & Tiralová, 2001; Curling, Clausen & Winandy, 2002). The result in this study on the mechanical properties on pine sapwood sampled every 6th month for 30 months indicates that impact bending (i.e. MOR and MOE) are small and not sensitive enough to predict the progress of decay. Mass loss was the best indicator showing the beginning of fungal attack.

Results obtained from the staple beds were compared with results from ENV12037 from Ultuna that was started simultaneously. It could be concluded that the progress of above ground colonisation of fungi is a slow process and that there is no significant difference between the speed of attack of the two set ups (staple bed and double layer). This means that the distance between the wood samples and ground contact in Ultuna is of no major importance.

Soft rot was the most abundant decay type found using light microscopy. This indicates that soft rot fungi may be of greater importance and challenge for the

wood preservation industry than is reflected by present standards and publications on wood durability testing of wood in above ground conditions.

Table 1. Showing the r^2 values for mass loss and degree of soft rot dependent on MOE, MOR, impact bending and mass loss during 30 months field exposure.

Test methods	Mass loss		Soft rot degree	
	field r^2	shadow r^2	field r^2	shadow r^2
MOE	0,05	0,33	0,40	0,30
MOR	0,38	0,02	0,87	0,31
Impact bending	0,15	0,16	0,19	0,06
Mass loss			0,70	0,68

Paper III

Paper III shows that the PCR method is sensitive for detecting early stages of decay. Results from a soil jar test sampled weekly were compared to results from light microscopy. The PCR method detected the fungus at an early stage (Table 2) of colonisation. This shows the potential for PCR-based methods for detection of fungal colonisation.

Different strains of the brown rot fungus *Coniophora puteana* and white rot fungus *Schizophyllum commune* were compared by using T-RFLP and sequencing of the ITS 1 and ITS 2 regions of the nrDNA gene cluster. There was no indication of overestimation of species due to intra-specific sequence variation for these species. It was not possible to discriminate between different strains; this makes the ITS 1 and ITS 2 region suitable for species identification using T-RFLP for these species.

Table 2. Results from the fungal colonisation test on pine sapwood. PCR results show that *C. puteana* was detected after two weeks inoculation. The positive results show how many of five replicates of *C. puteana* that were amplified. For *P. placenta* there was one replicate and positive results are therefore indicated by yes or no. Microscope results show absence or presence of the fungus.

Week	*Coniophora puteana*			*Postia placenta*	
	Detected by PCR	Positive results	Detected by microscope	Detected by PCR	Detected by microscope
2	Yes	1	No	Yes	No
3	Yes	5	No	No	No
4	Yes	5	No	Yes	No
5	Yes	5	No	No	No
6	Yes	4	No	Yes	No
7	Yes	2	Yes	Yes	Yes
8	Yes	2	Yes	No	Yes
9	Yes	3	Yes		

Paper IV

Paper IV describes the external and internal fungal flora of pine sapwood in field exposed specimens at 6 different locations in Europe. The pine specimens had been exposed for 3-4 years in the field before examination. The fungal DNA was isolated directly from the wood specimens and the community was determined using PCR followed by cloning, sequencing and T-RFLP.

The study showed that soft rot was again the most abundant decay type as determined by both molecular methods and visual observations in the field. The most common fungus found in the study was *Coniochaeta ligniaria* ((Grev.) Cooke), a soft rot fungus. In total, 62 different fungal species were found, mainly belonging to the ascomycetes. The number of fungal species did not correlate with decay intensity, rot type or characteristics of the test sites. Flattening of the species accumulation curve (Figure 8) indicates that our sampling approach was sufficient for a large proportion of the fungi present, both as a total and also for drilled and surface samples taken separately.

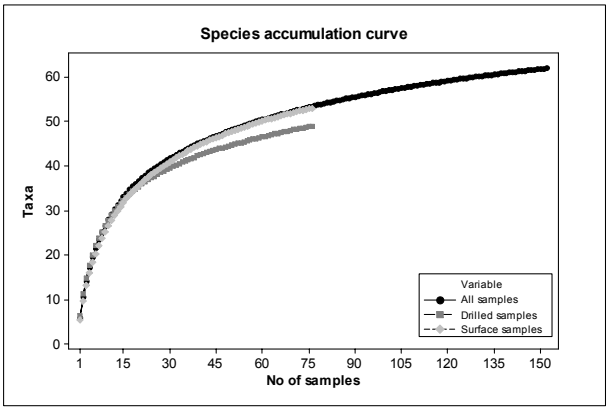

Figure 8. The graph shows a species accumulation curve for all samples, drilled samples and surface samples in paper IV. The curves show that the sampling approach adopted was adequate.

Cloning made of selected PCR products gave a good impression of what could be expected in these field-exposed samples. The identification of species in this study was based on the information of T-RFLP profiles obtained by cloning selected PCR amplicons and from T-RFLP profiles obtained from the Department of Wood Sciences fungal herbarium.

No single geographic and climatic parameter observed in this study appears to be decisive for number of fungal species or decay intensity. Instead, a combination of all parameters prevalent at a test site affects both the number of fungal species detected and decay intensity (Table 3).

Table 3. p-values for the relation between number of species at different test sites as a whole or for height above sea level, average temperature and precipitation.

	Drill p	Surface p
Test site	0.0043**	0.0004***
Height above sea level	0.6053	0.0936
Average temperature	0.6538	0.1445
Precipitation	0.2933	0.3594

** 1% significance ***0,1% significance

Discussion

Testing and evaluation of wood

An overview on the testing and evaluation of wood in above ground conditions showed that previously most accelerated tests were either conducted in fungal cellars or placed closer to the ground than what the standard prescribes. In this thesis, staple bed and double layer tests have been used as an attempt to shorten the duration of field testing. Results (papers II and IV) indicate that accelerated tests placed closer to the ground do not shorten the duration of the test in Nordic conditions. The test site location is more decisive for the duration of the test. This means that characterising different test sites for deterioration types (e.g. bacterial, soft rot, brown rot or white rot) and using a mixture of these sites would be more informative than different kinds of accelerated tests. The reason for this may be that the infestation and establishment of fungal communities on wood is not dependent on the height above ground. Studies by Carey (2002a, b) showed poor correlation between the detection and onset of decay, which suggests there may be available spores and smaller establishment of decay fungi for some time before deterioration of wood occurs. In this thesis soft rot fungi were the most abundant rot type, for both staple beds and double layer tests (papers II and IV).

It is a common misconception that soft rot fungi only occur in very wet wood (Blanchette et al., 1990; Daniel & Nilsson, 1998). This work also confirms this erroneous belief (papers II and IV). The most common soft rot fungi found in the double layer test was *Coniochaeta ligniaria* also known as *Phialophora hoffmanni* and *Lecythophora hoffmannii*. In the staple bed test, soft rot was the major rot type detected by microscopy, and once again *L. hoffmannii* was detected using rDNA sequencing. *Phialophora spp*. are known to cause both soft rot and discolour wood. Soft rot fungi can tolerate large moisture content fluctuations, and have been found in wood that has remained dry for long periods with only intermittent wetting (Findley, 1965; Blanchette et al., 1990; Blanchette, 2005). Soft rot fungi are considered to have their main impact in the early colonisation of wood (Clubbe, 1980; Eaton & Hale, 1993). Clubbe (1980) observed that soft rot fungi are displaced quickly by non-decaying and later decaying basidiomycetes in untreated pine. However, this may not always be the case even though they occur in certain ecological niches (wood substrates) where these fungi can be active due to a lack of competition (Daniel & Nilsson, 1998). Presumably soft rot fungi are only replaced if the substrate is favourable for decaying basidiomycetes; otherwise, soft rot fungi remain the essential wood degraders. In this thesis, the duration of the test was too short to study the replacement of detected soft rot fungi. Compared with brown- and white rot fungi, soft rot fungi are considered to have a much slower decay (Käärik, 1974). They can also degrade wood without additional nutrients, but it has been shown that addition of nitrogen significantly increases the rate of degradation (Findley, 1965; Blanchette et al., 1990; Daniel & Nilsson, 1998).

It has been confirmed by a number of studies (Wilcox, 1978; Reinprecht & Tiralová, 2001; Curling, Clausen & Winandy, 2002) that the development of decay correlates well with a decrease of the mechanical properties of wood. In the main, these results are from laboratory tests (Nicholas & Crawford, 2003) or tests in ground contact (Henningsson et al., 1975). It appears to be more difficult to correlate a change in the mechanical properties of wood with the degree of soft rot attack in above ground conditions for early stage of exposure (2-3 years). Earlier test results (Eaton & Hale, 1993) revealing that a mass loss of only a few percent can drastically decrease the impact bending strength, were not confirmed in this thesis. The impression was rather that determination of mass loss best showed the early stages of fungal deterioration (paper II). Results of strength loss in the staple bed test were compared with results obtained by the standard lap-joint test exposed in the field at the Swedish University of Agricultural Sciences in Uppsala. A lap-joint test that was most comparable with the staple beds was started simultaneously in 2003. This lap-joint test showed relatively rapid attack of decay fungi and a median rate of 1 (according to ENV 12037) was recovered after 2 years exposure. The results from both lap-joints and staple bed samples support the conclusion that the above ground colonisation of wood is a slow process and in the case of exposure in Uppsala, no significant differences in the speed of attack could be revealed between the standard and alternative accelerated methods. These results also showed that the distance from the test samples to the ground for the conditions of Ultuna was of no significant importance.

Limitations of the T-RFLP method

The ecological application of molecular methods such as T-RFLP and other community profiling methods is not without problems. Some problems are related to band resolution and the detection of PCR artefacts, while others are associated with subsequent analysis. PCR artefacts include: 1) preferential amplification; 2) non-specific products; and 3) hybrid amplification (chimeras). In preferential amplification replication, one template is favoured, resulting in a skewed representation of species abundance (Kitts, 2001; Allmér, 2005; Mitchell & Zuccaro, 2006). Non-specific products or so called pseudo TRF can appear in the analyses (Egert & Friedrich, 2003; Mitchell & Zuccaro, 2006). Egert and Friedrich (2003) suggested that the presence of single stranded DNA is the cause of the formation of pseudo TRF. They also found that the restriction enzyme Taq I was not affected by the pseudo TRF formations. Looking for products that contain mixed and conflicting signals can recognize chimeric molecules. Biases in PCR caused by impurities in the sample, or the relative proportion of reaction constitutes (chemicals, target and non-target DNA) may also affect the outcome. Many of these artefacts can be eliminated by careful optimisation of the replication reaction prior to community profiling (Mitchell & Zuccaro, 2006).

With band profiling methods, it is often assumed that a single band represents a single species, but this is not always true. The reason may be both biological and physical. Some fungi may contain multiple genetically distinct nuclei or have heterogeneous rDNA clusters. The amplification of the rDNA from such a fungus will therefore generate multiple bands for the same species. It is therefore

important to obtain accurate identities for each band, so the PCR artefacts and unresolved bands do not complicate the analysis. Demonstrating that a sequence from an environmental sample is authentic is best achieved by matching with known species or isolates from the same environment. If this is not possible, the sequence should be checked for mixed signals and its frequency of recovery monitored. In general, molecular cloning should accompany the T-RFLP analysis to provide templates for comparison as conducted in paper V.

Toljander (2006) tested the effect of dilution on template prior to PCR and T-TFLP on the relative abundance of restriction fragments. There was no relation between the peak heights and dilutions indicating that great caution should be taken translating T-RFLP profiles to relative abundance of species in environmental samples.

In general, T-RFLP is both a reproducible and precise tool. T-RFLP can be used to study colonisation of a substrate by multiple sampling within the same local, and also for studies of interactions between fungi over time. Studies in this thesis have shown that it is possible to use T-RFLP as a tool for fungal identification directly from wood samples exposed in the field.

Fungal detection and colonisation

Allmer (2005) found differences in species composition and richness by comparing mycelial isolation, ITS T-RFLP and sporocarps on wood stumps, branches and tops. This demonstrates that the detected fungal community is dependent on the detection and identification methods used. Molecular methods can be used to detect rare species that cannot be cultivated and also to identify fungal species that are morphologically difficult to differentiate. Sporocarp identification has the limitation that the fungi need to form fruit bodies before they can be identified. Mycelial identification has limitations in that some genera are morphologically difficult to discriminate between.

Clubbes (1980) study of colonisation and succession of fungi in *Pinus sylvestris* and *Betula pendula*, showed the first decay fungi to be soft rot fungi like *Phialophora spp*., *Trichocladium opacum*, *Humicola* and *Drechslera dematioides*. The following Basidiomycetes were *Sistotrema brinkmanni*, *Sterum hirstum* and *Bjerkandera adusta*. Choi, Ruddick and Morris (2003) found mainly *Phialophora spp* in CCA treated decking. More fungi that can cause soft rot were also isolated like *Cephalosporium, Exophiala* and *Rhinocladiella*. The basidiomycetes isolated were *G. sepiarium*, *G. trabeum* and *S. brinkmanni*. In this thesis the soft rot fungus *C. ligniaria* was most frequently isolated. *Coniochaeta* species often have a *Phialophora*-like anamorph (Hawksworth & Yip 1981) and is a telemorph of *L. hoffmannii* (anamorph). *L. hoffmannii* is synonymous with *P. hoffmannii* and has been isolated in association with wood decay (Käärik, 1974; Hawksworth & Yip, 1981; Savonmäki, Salonen & Ruokola, 1992; Allmer, 2005). Another frequently occurring fungus identified in this thesis was *Exophiala spinifera* also known under the synonyms *P. spinifera* and *Rhinocladiella spinifiera*. The Basidiomycetes that

have been found in this thesis using T-RFLP analysis were *Sistotremastrum sp., A.. serialis, G. trabeum* and *P. placenta*. These species are expected according to the fungal community that inhabit construction wood exposed outdoors (Clubbe, 1980; Richardson, 1993; Schmidt, 2006), while the unpredicted part was the strong abundance of soft rot. This may be due to the short duration of the exposure before fungal identification. If fungal identification will be performed again on the field test exposures after a number of years, the fungal community will probably have shifted towards a greater number and more frequent isolation of basidiomycetes species. Results from this thesis however, show that soft rot fungi have a significant effect in above ground conditions concerning mass loss and therefore in the early deterioration of wood.

This work has showed that it is possible to use molecular methods to detect and identify wood decaying and discolouring fungi. Molecular methods are useful in that they can detect and identify fungal species that cause deterioration or discolouration fast, precisely and directly from the infested substrate.

Conclusions and future perspectives

The main conclusions are:

- PCR-based methods like T-RFLP applied directly on wood samples can be used for identification of wood decay and discolouring fungi.
- Soft rot fungi appear to be more abundant in above ground conditions than is reflected by present literature.
- The degree of soft rot correlated well with mass loss and was a better measurement for early stages of decay than impact bending, MOR and MOE.
- There were no straightforward correlations between the identified fungi, decay intensity, strength loss and mass loss or single environmental conditions (e.g. average temperature, precipitation and altitude).
- The speed of field testing of samples above ground is not dependent on the height above ground.

The future for using molecular methods for the testing of wood is promising. There is a need for more data considering the fungal population and colonisation of construction wood. This thesis offers only an inspiration of what could be explored in the field.

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Summary in Swedish

Trä och träskadesvampar

Trävirke har används i olika konstruktioner under många tusen år, mycket beroende på att det är ett starkt material i förhållande till sin vikt. Hållfastheten kan dock kraftigt försämras av kvistar, snedfibrighet och andra oregelbundenheter i virket. Virke som har en fuktkvot över ca 25-30% kan angripas och brytas ned av träförstörande mikroorganismer, främst rötsvampar.

Träskadesvampar kan delas in i mögelsvampar, blånadssvampar och rötsvampar. Dessa grupper skiljs åt främst genom deras sätt att angripa, tillväxa och överleva på och i virket. Mögelsvampar växer på virkesytan och är mer beroende av omgivande fukt och temperatur än virkets. Mögelsvamparna angriper inte vedens celler och har ett ofärgat mycel (svampens rötter), men ofta stora mängder med färgade sporer som ger ett missfärgat intryck. Blånadssvamparna växer in i virket, men angriper och förstör inte cellerna i någon större omfattning. Mycelet är färgat i grönt, blått, brunt eller svart, vilket är orsaken till den missfärgning som svampen åstadkommer. Rötsvamparna växer inne i veden och bryter ned cellerna, vilket gör att virket ruttnar. Rötsvampens mycel är ofärgat och kräver vatten för att kunna utvecklas, därför kan inte torrt trä ruttna. Förmågan att motstå angrepp från rötsvampar kallas rötbeständighet och varierar starkt mellan olika träslag samt mellan ytveden (splintved) hos barrträd och kärnveden. På lång sikt bryts dock allt fuktigt trä ned av mikroorganismer som en del i naturens kretslopp. Rötsvampar kategoriseras efter dess sätt att bryta ned veden och delas in i brun- och vitröta samt soft rot.

Fältförsök

Fältförsök används för att visa hur rötbeständigt olika träslag eller träskydd är när de utsätts för åverkan som liknar den miljön de kommer att användas i. Det finns några europeiska standard tester, EN 252 för trä i markkontakt, EN 330 och ENV 12036 för trä ovan mark. I den här studien har modifieringar av dessa standarder använts för att påskynda rötprocessen men ändå bevara den åverkan som finns i fälten (främst fukt och svampsporer). I den ena varianten har ett dubbelt trädäck (double layer se figur 4) byggts upp 200 mm ovan mark, i den andra varianten ligger virket direkt mot marken och är staplad sju lager upp (stapel bädd se figur 4). Enligt den Europeiska standarden bestäms rötgraden av virket i fält genom en femgradig skala (0-4) med hjälp av en kniv. Kniven förs in i virket och bryts lätt uppåt. Beroende på hur fibrerna bryts av och hur utbredd rötan är graderas den enligt den femgradiga skalan.

Artbestämning av svampar

Traditionellt har svamparna bestämts genom att isolera svamparna på olika näringsmedium. Svampen bestäms sedan med hjälp av dess morfologi (utseende). En del rötsvampar bildar fruktkroppar och har kunnat artbestämmas, men en del av svamparna bildar inte fruktkropp i laboratorieförhållanden och blir därför svårare

att bestämma. Artbestämningen baserar sig då på dess mycel, svampens ”rötter”. Ibland är olika arters mycel så lika att de inte kan särskiljas. Att odla på olika medium är tidsödande och det kan ta månader innan svampen har utvecklat karaktäristiska kännetecken för arten och kan artbestämmas.

För att få reda på vilken svamp som rötar trävirket kan virket analyseras med molekylära metoder. För att artbestämma svampen används dess DNA som jämförs med DNA från redan kända och bestämda svampar. Svampens DNA går att påvisa och isolera redan där det finns väldigt små mängder av svampen eller svamparna. Metoden är också snabb jämfört med den traditionella odlingstekniken.

Syfte med avhandlingen

Syftet med avhandlingen var att se om det är möjligt att artbestämma träskadesvampar i virke snabbare, effektivare och specifikare med molekylära metoder än med den traditionella odlingsmetoden, samt att undersöka sambanden mellan den omgivande miljön, den biologiska nedbrytningen och de påvisade svamparna.

Resultat

Resultaten visar att det går bra att artbestämma träskadesvampar molekylärt. Det går att artbestämma rötsvampar direkt från träprover utan att renodla eller klona[*] svamparna innan analys. Metoden baserades på att en del av svampens DNA mångfaldigas så att en tillräcklig mängd erhålls för analys. Den fortsatta analysen kan vara att den mångfaldigade delens sekvens[**] bestäms, eller att om det finns fler okända svampar i ett prov, att skapa en T-RFLP profil[***] för provet. Med en T-RFLP profil kan en eller flera svampar artbestämmas samtidigt i ett prov.

Resultaten ger inga entydiga samband mellan de artbestämda svamparna, biologisk nedbrytning och omgivande miljö.

Användbarheten av resultaten

Den molekylära metoden har fungerat bra och kan användas i praktiskt bruk där träskadesvampar behövs artbestämmas. Det kan exempelvis vara för att påvisa frånvaro av rötsvampar, göra rätt sanering, försäkringstvister osv.

[*] Att klona innebär att man åstadkommer genetiska kopior av en individ.
[**] En sekvens beskriver i vilken ordning DNA molekylerna sitter i.
[***] T-RFLP profilen är baserad på svampens DNA sekvens.