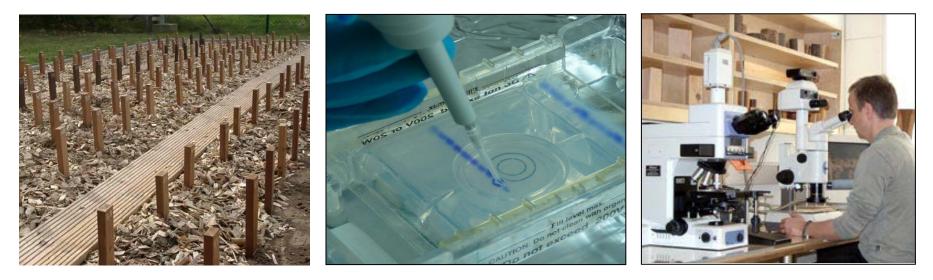


Diversity of wood decay fungi in test fields across Europe



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Funded by the German Ministry of Economy and technology



Bundesministerium für Wirtschaft und Technologie

aufgrund eines Beschlusses des Deutschen Bundestages



Background

- Fungal diversity
 - In buildings: about 30 typical and well known fungi (30 % to 50 % dry rot fungus),
 - Much higher diversity of species in outdoor wood applications (UC 3/4),
 - From worldwide estimated 1.5 to 3 million fungal species , only 200,000 have been described yet (Hawskworth 2003),
 - Knowledge about mechanisms of wood degradation and involved fungal species insufficient,
 - Better understanding of wood decay and involved fungi important for improvements in wood protection.



Motivation



- New methods for microbial community analysis
 - Classical methods are increasingly supplemented by molecular biological methods, most important DNA analysis,
 - Detection of fungi in all stages of development; no need for specific morphological characteristics or cultivation,
 - Analysis of the full range of species within an habitat,
 - Revision of the taxonomy and nomenclature of fungi by genetic data,
 - DNA based analysis is reliable and objective and can be standardized.





- Investigation of diversity and succession of wood decay exposed in ground contact:
 - Different wood species,
 - Different test fields / locations,
- Development of molecular diagnostic methods,
- Providing genetic reference data.

Tubaria hiemalis around a scots pine sapwood stake in the test field Hamburg Photo: E. Melcher 10/2010



Field exposure of wooden stakes





Collaboration with other european test laboratories:

- Bordeaux (FCBA)
- Hamburg (vTi)
- Udine (Catas)
- Dresden (IHD)
- Poznań (ITD)

Exposure conditions:

- in ground exposition according to EN 252
- ^o Stakes: (50 x 25 x 500) mm³,
- scots pine sapwood (*Pinus sylvestris*) and beech (*Fagus sylvatica*),
- Sampling at intervals of 6 months,
- Analysis of 3 stakes per wood species, location and exposure time.



Sampling

- Surface sampling
 - Taken from visible infested places at the whole specimen,
 - Distinguished by three areas: above ground (A), earth/air passage area (B) and in ground (C),
 - No defined number of samples.





Surface sampling for DNA analysis

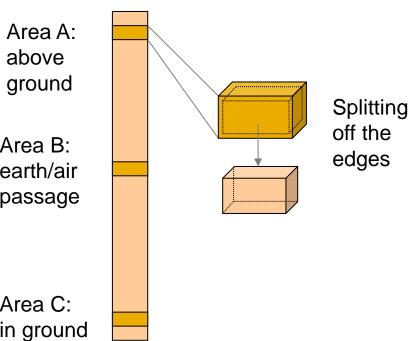
Sampling



- Sampling from the interior of specimens
 - Systematic sampling from defined areas of each specimen,
 - Resulting in defined number of samples from each specimen and area.



Specimens from Udine after 18 month exposition



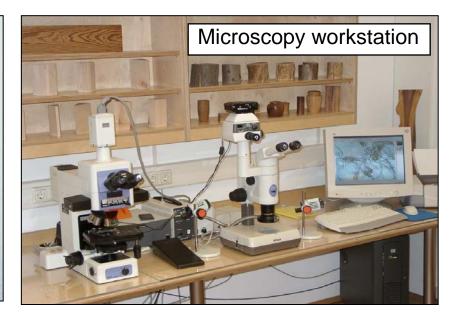
Schematic illustration of interior sampling



- Methods:
 - Reflected light microscope: investigation in different areas with visible infestation at magnifications of 7.5 ... 110-fold,
 - Transmitted light microscope: investigation of single locations at higher magnifications (up to 750-fold),
 - Preparation of thin cross or longitudial sections of infested areas using microtom device or blade.



Preparation of thin sectiones using a blade





Conventional analysis

- Identification of fungi by
 - Investigation of morphological characteristics at fruiting bodies and spores, surface and substrate mycelium, strands;

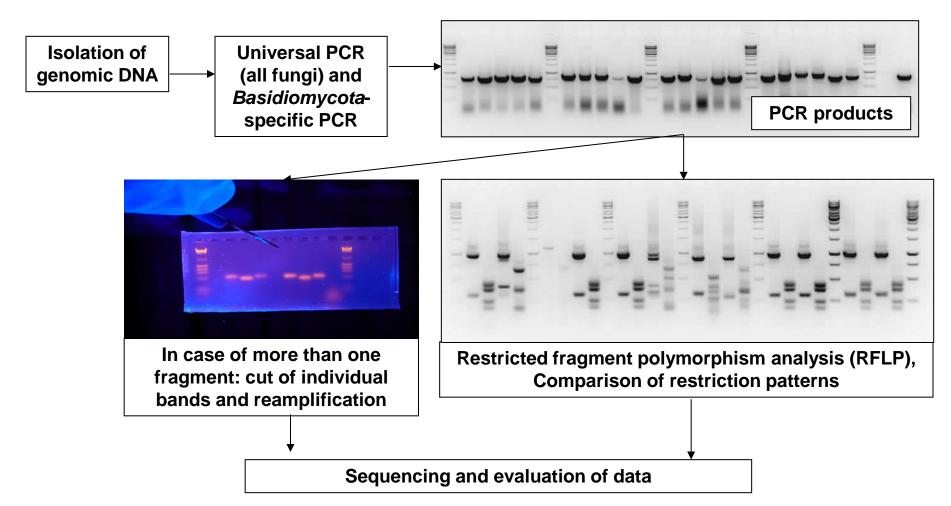


- Investigations of decay patterns in damaged wood (e.g. cavities caused by soft rot fungi);
- Specification of sampling spots for DNA analysis after conventional investigation and documentation, restricted to different fungi and infestation patterns, respectively.



DNA-Analysis

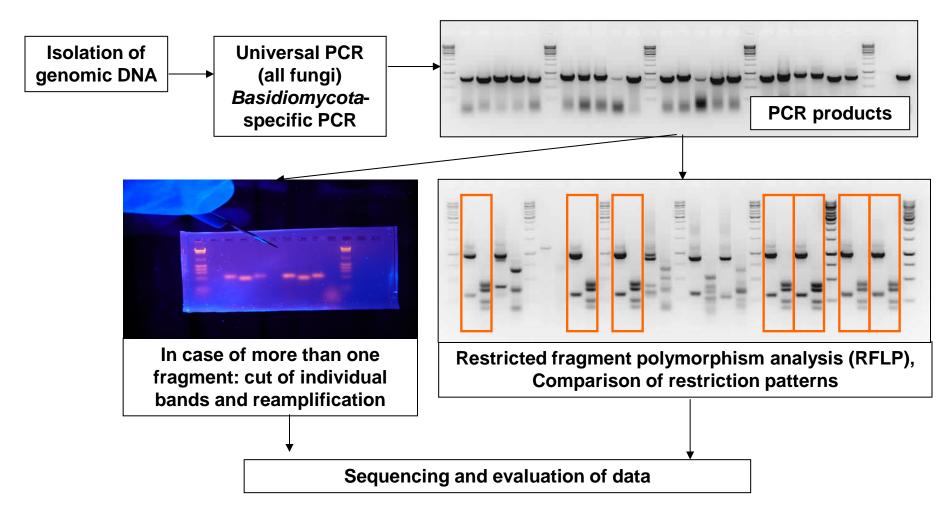
Workflow of molecular identification of fungi





DNA-Analysis

Workflow of molecular identification of fungi

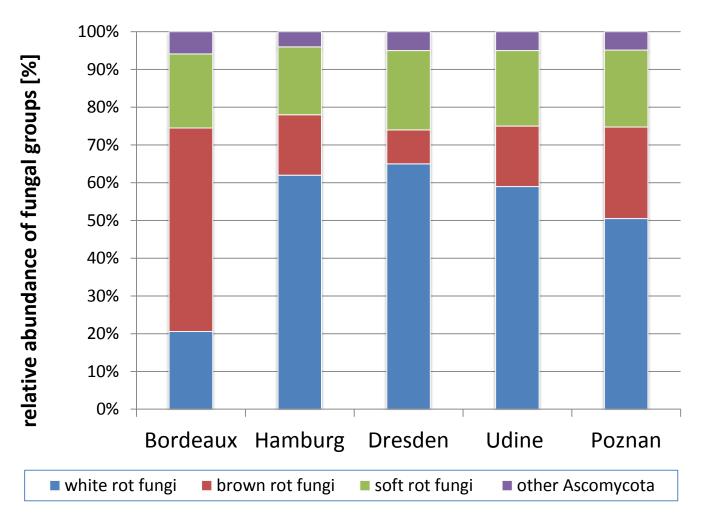




- Summarized results
 - Evaluation of 150 stakes from 5 locations (more than 1500 individual samples for DNA analysis),
 - Identification of 98 fungal species from 77 genera,
 - No clear species differentiation in 43 cases, mainly Ascomycota,
 - On average 25 different fungal species per test field and exposure time,
 - Ascomycota (soft-rot fungi) not completely detected due to the limits of the used methods,
 - A general increase of fungal diversity with increasing exposure time was not observed.



Types of wood degradation



Dominant fungal species at different locations

Test field	Scots pine sapwood	Beech
Hamburg (11)	Cyathus striatus, Cylindrobasidium evolvens, Helicosporium sp., Hypholoma fasciculare , Peniophora sp., Psathyrella sp., Scopuloides hydnoides, Sistotrema brinkmanii	Armillaria sp. , Bjerkandera adusta, C. striatus, C. evolvens, Exidia glandulosa, H. fasciculare , Psathyrella sp., S. hydnoides, S. brinkmanii
Dresden (9)	C. striatus , Helicosporium sp., Resinicium bicolor, S. brinkmanii	B.adusta, C. striatus , E. glandulosa, Hypochnicium sp., S. brinkmanii, Trametes versicolor
Udine (11)	Helicosporium sp., Oxiporus sp. , Peniophora sp., Schizophyllum commune, Sistotrema sp.,	B.adusta, C. stercoreus, E. glandulosa, Oxiporus sp ., Stereum sp., T. versicolor
Bordeaux (8)	Coniophora sp., Mycena sp., Peniophora sp., Sistotrema sp. , Leucogyrophana sp.	Postia ptychogaster, Serpula himantioides, Stereum hirsutum
Poznań (7)	H. fasciculare , Postia sp., Sistotrema sp.,	B.adusta, E. glandulosa, H. fasciculare , Mycena sp., Psathyrella sp.



Selected dominant fungal species: Hypholoma fasciculare

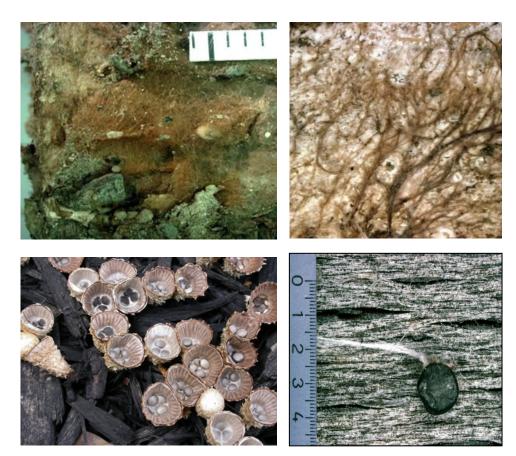




- Frequent occurrence in Hamburg and Poznań,
- found on the majority of stakes (pine and beech), also in the interior,
- white rot fungus,
- widespread saprophyte on dead hardwood and softwood within Europe.



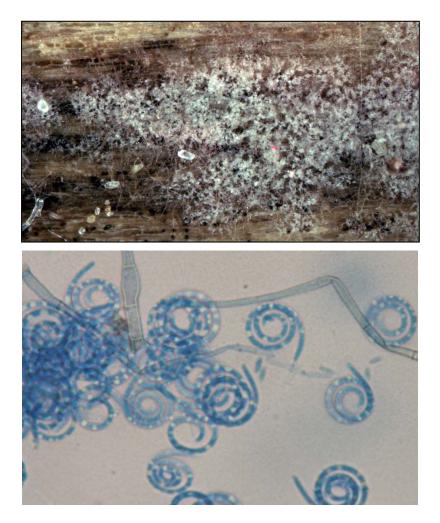
• Selected dominant fungal species: *Cyathus striatus, C. stercoreus*



- Frequently found in Dresden, Hamburg, Udine,
- Only external infestations of beech but strong attack in the inside of pine sapwood,
- Associated with intense white rot,
- Common saprophyte of deciduous and coniferous trees,
- Formation of red-brown surface mycelia (also strands) and cupshaped fruiting bodies with spore carriers (peridioles).



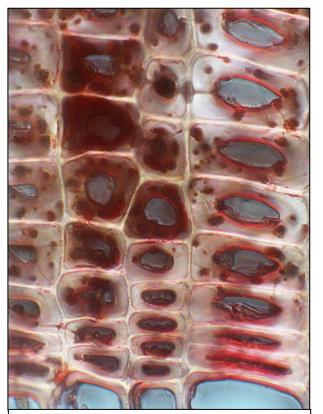
• Selected dominant fungal species: *Helicosporium* sp.



- Frequent occurrence at the sites of Dresden, Hamburg, Udine, Poznań,
- Exclusively on pine sapwood, exterior and interior,
- Always associated with soft rot (underneath),
- Blue-grey surface mycelia with characteristic helical spores,
- DNA analysis on species level by rDNA-ITS sequencing failed due a lack of reference data.



Microscopic detection of soft rot



Cross section, M 1000:1; Soft rot cavities in the late wood cell walls of tracheids



Radiale section, M 600:1; diagonal directed soft rot in the tracheids wall of early wood

- Thin cuts of pine wood in cross and longitudial direction,
- Detection of soft rot in beech and scots pine sapwood stakes at all locations,
- Differentiation of fungal species often failed due to insufficient reference data base,
- e.g. Heliotales spp.,
 Sordariales spp.,
 Phoma spp.

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Results

Wood decay by termites



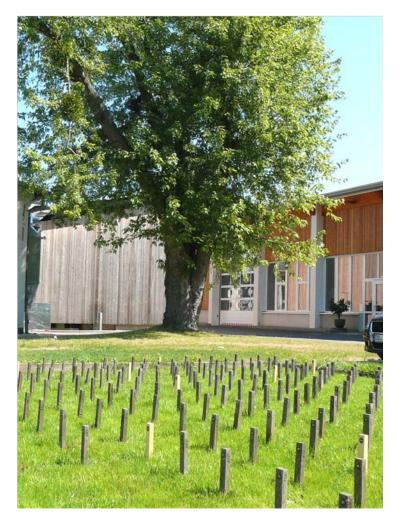
- Special situation in Bordeaux (Île d'Oléron) because of massive infestation by termites (*Reticulitermes* santonensis),
- Found at pine and beech wood,
- Accelerated breakage of stakes caused leak of fungal diagnostic results for longer exposure time (3 and 3,5 years).

Summary



- White and soft rot fungi are the predominant type of decay exepting the field near Bordeaux.
- A total of 98 different fungal species from 77 genera were identified.
- In 43 cases an identification on species level failed due to lack of reference data or limits of used analysis methods.
- 25 different fungal species were found on average at each location and exposure time.
- A general increase of fungal diversity with exposure time was not observed.
- At each test field 7-11 dominant fungal species were found.
- The majority of the identified dominant fungal species are not commonly known as decomposers of used wood.





Thank you for your attention!

Presented work was part of a project, funded by the German Ministry of Economy and technology (Reg.-Nr. VF090010).