SNS Forest Pathology Meeting

Hyytiälä, 26-29 August 2007

PATHCAR-project
Finnish Forest Research Institute
University of Helsinki

A short summary of the meeting.

The meeting was held in a forestry field station of the University of Helsinki. Altogether six oral sessions were composed of 25 presentations. In addition there was a poster session, two stops at experimental sites, and a field trip to a national park. During the meeting, many important novel scientific and practically useful aspects were discussed.

The first session was dealt with needle and shoot diseases of conifers. The presentations highlighted developments in the understanding of population dynamics and ecological behaviour of fungal tree diseases. This we consider to be of high importance under the changing climate in future. It was obvious from the presentations in this session that special attention should be put in using correct provenances in forest regeneration.

The second session composed of presentations about the possibility to use *Chondrostereum purpureum* in controlling hardwood sprouting. The experimental sites visited were also related to this topic. Overall, the results presented showed high potential of the approach, but further work is still needed.

The third session focused on the emerging sequence of the genome of *Heterobasidion annosum* and other aspects of decay. It seems that a new era based on unforeseen understanding of the biology of *Heterobasidion* root and butt rot is appearing. The importance of this fungus in predicting climate change was highlighted with results showing that decay is an extremely important factor in wind-falls. This equally underlines the importance of research on practical ways of controlling *Heterobasidion* in boreal forests.

In session four, the research on new emerging diseases was presented. The ash dieback in southern part of the region is very serious, this is most probably caused by a new fungal species. New information is urgently needed on this problem. On the other hand, a serious epidemic of oak decline seems to be levelling down, and the future of oak forests in Nordic/Baltic countries seems bright. However, new diseases (such as *Phytophthora* spp.) and novel uses of trees (energy forests) demand continuous research on forest pathology.

In session five, it was shown that spruce cone rusts have turned out to be of high importance in reducing seeds, especially in good cone and seed years. *Laetiporus sulphureus* s.l., the sulphur polypore or chicken of the wood, consists of a species complex with at least seven well separated phylogenic clades world wide; now separated with the aid of molecular approach. Analyses of decay on urban trees have identified some of the most dangerous causative fungi. The results of this study is expected to help the practical people in estimating the correct sanitary decisions in city parks. On conifers, the needle trace method can be used to understand the causes of past epidemics.

In session six, there were presentations on the role of secondary compounds in tree defence against pathogens. Also the use of mycorrhizal inoculations to increase growth and survival of conifer trees in abandoned farmland was highlighted. However the establishment of plants and fungal community formation in root systems seemed to be governed mainly by environmental factors. In the last presentation it was learned that in Oslo region the Dutch elm disease appears not to be efficient enough to completely destroy elm trees, but equilibrium between the disease and regeneration of elm populations seems to take place.

Program for the SNS meeting in Hyytiälä, Finland

26. - 29.8.2007

Sun 26.8.

16.00 -	Arrival and registration,
19.00	Welcome, get together
20.00	Sauna, women
21.00	Sauna, men

Mon 27.8.

7.00-8.30	Breakfast
8.50-9.00	Opening words
9.00	Oral session 1
11.30	Lunch
12.30	Visit to an experimental site (sprout control)
13.30	Oral session 2
14.30	Coffee break
15.00	Oral session 3
17.30	Dinner
18.00	Sauna, women
20.00	Sauna, men

Tue 28.8.

7.00 - 8.30	Breakfast
8.30	Oral session 4
11.30	Lunch
12.30	Departure to field trip
13.00	Stop in an experimental site (birch pruning)
14.30	Coffee break in Helvetinkolu
17.00	Return to Hyytiälä
18.00	Banquet including fish smoking

Wed 29.8.

7.00 - 8.30	Breakfast
8.30	Poster session
9.30	Oral session 5
11.30	Lunch
12.30	Oral session 6
14.00	Business meeting
14.30	Coffee break
15.00	Walk to the SMEAR-station
16.00 -	Departure

Scientific program

Oral session 1: Monday 9.00 - 11.30
Chair: Fred Asiegbu

9.00 Michael Müller (Finland): Variation of *Lophodermium piceae* within the distribution area of *Picea abies*
9.30 Märt Hanso (Estonia): Retrospective survey of the *Lophodermium* needle cast epidemics in Estonia
10.00 Jarkko Hantula (Finland): Diversity, host range and population genetics of *Gremmeniella abietina* viruses
10.30 Seppo Nevalainen (Finland): Spatial and temporal variation of *Gremmeniella abietina* in Finland
11.00 Martti Vuorinen (Finland): Resistance differences between pine provenances against shoot and needle cast diseases

Oral session 2: Monday 13.30 - 14.30
Chair: Talis Gaitnieks

13.30 Remigijus Bakys (Sweden): *Chondrostereum purpureum* as a biocontrol agent against undesired sprouting of deciduous trees
14.00 Henna Vartiamäki (Finland): Genetic diversity of *Chondrostereum purpureum* in Finland and Lithuania

Oral Session 3: Monday 15.00 - 17.30
Chair: Iben M. Thomsen

15.00 Åke Olsson (Sweden): *Heterobasidion* genome project
15.30 Jan Stenlid (Sweden): Sequencing the *Heterobasidion* genome
16.00 Fred Asiegbu (Finland): In vivo differentiation of *Phlebiopsis gigantea* hyphae from *Heterobasidion annosum* at barrage zones using confocal microscopy
16.30 Jonàs Oliva (Sweden): Urea protection against *Picea abies* butt rot: results of a 15 year experiment
17.00 Ylva Persson (Sweden): Do bark and wood-boring insects in mechanically created high stumps of Norway Spruce facilitate the establishment of rot fungi?

Oral Session 4: Tuesday 8.30 - 11.30
Chair: Halvor Solheim

8.30 Pia Barklund (Sweden): Ash decline in Sweden
9.00 Iben M. Thomsen (Denmark): Ash dieback in Denmark
9.30 Kestutis Grigaliunas (Lithuania): Forest sanitary problems in the period of 1996-2006 in Lithuania
10.00 Arja Lilja (Finland): *Phytophthora inflata*, a new alien species in Finland
10.30 Merje Toome (Estonia): Willow leaf rust in Estonian energy forest plantations and the factors influencing its occurrence
11.00 Vaidotas Lygis (Lithuania): Investigations on oak decline in Lithuania

Poster session: Wednesday 8.30-10.00

Oral session 5: Wednesday 9.30 - 11.30
Chair: Jan Stenlid

9.30 Juha Kaitera (Finland): Some ongoing research and future research interests with spruce cone rusts
10.00 Rimvys Vasaitis (Sweden): *Laetiporus sulphureus s.l.*: ecology and genetic diversity
10.30 Minna Terho (Finland): Wounds and decay on urban trees
11.00 Rein Drenkhan (Estonia): Alterations of Scots pine growth and needle characteristics after abrupt winter onsets: a retrospective analyses using the needle trace method (NTM)

Oral session 6: Wednesday 12.30 - 14.00
Chair: Märt Hanso

12.30 Johanna Witzell (Sweden): Secondary chemistry of poplars in relation to natural enemies
13.00 Audrius Menkis (Sweden): Impact of mycorrhizal inoculation on establishment and growth of conifer seedlings on abandoned farmland
13.30 Halvor Solheim (Norway): Dutch elm disease in Norway (15 years after the sanitary program stopped)

Session 1. Needle casts and shoot diseases of conifers

Are subpopulations of European *Lophodermium piceae* genetically differentiated?

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Lophodermium piceae is an ubiquitous endophytic inhabitant of Norway spruce needles (*Picea abies)*. It can generally be found in the majority of older (> 3 yr) needles of single trees and it may be one of the most numerous fungi in spruce forests. The fungus is transmitted by aerial spread of ascospores, which are formed on dead needles still attached to twigs in the tree crown or on fallen senescent needles. Locally, *L. piceae* is a highly diverse fungus and it is difficult to find identical (characterized by DNA markers) isolates even within a single needle. The aim of this study was to examine the degree of differentiation within and among European subpopulations separated by various distances and geographical barriers. For this purpose, samples of subpopulations (including > 10 isolates/subpopulation) were collected along a north-south transect stretching from the northern timberline in Finnish Lapland to the southern border of the distribution area of Norway spruce in northern Italy. Differentiation between *L. piceae* populations was determined from DNA sequences of three genetic markers. One of the markers was the internal transcribed spacer (ITS) of the ribosomal DNA and the other two (LP1 and LP2) were sequence characterized amplified regions (SCAR) found in *L. piceae*. Preliminary results including sequences of Finnish, Swiss and Italian isolates show low differentiation among populations. According to analysis of molecular variance the among population variation was 1%, 5% and 0% in ITS, LP1 and LP2 markers, respectively.

Retrospective survey of the *Lophodermium* needle cast epidemics in Estonia

Märt Hanso and Rein Drenkhan
Estonian University of Life Sciences, Institute of Forestry and Rural Engineering

Comprising: 1) all the data, documented in the items of published literature as the *Lophodermium* needle cast epidemics in Estonia, 2) all the serious (with >50% loss of foliage) *Lophodermium* needle cast cases, diagnosed by the elder author (M. Hanso) in the Estonian forest nurseries during 1972-1985, 3) the NTM (needle trace method) data, indicating the needle losses of young age Scots pines in five different forest stands of south-eastern Estonia and covering most of the period between 1884 and 2004; and 4) meteorological data, enabling to select out the most congenial years for the rise of a new epidemic inside that long period, and, after that juxtaposing the documented and proposed epidemic years (regarding data from 1. to 3., cf. above) with the years (regarding data from 4., cf. above), supposed as congenial to the pathogen (wet summer and mild winter before the pointer year, this way concerning the meteorological peculiarities, which, according to the literature, have to predispose the start of a new epidemic), it was concluded, that in most (but not in all!) documented and/or proposed epidemic years the dependence from the predisposing meteorological conditions was statistically significant.

It means that 1) weather conditions, predisposing an epidemic are important, but 2) there is still room for additional research efforts for the explanations of the rise of a new epidemic, incl. research on the molecular level, e.g. for the investigations of the improvement of the pathogens virulence between two-in-order epidemics.

Epidemics of *Lophodermium* needle cast disease of Scots pine were more seldom in plantations than in forest nurseries. A preliminary list of epidemics of *Lophodermium* needle cast from 1884 until 2004 (incl. both, documented and proposed) was included into the presentation.

Taxonomic diversity of viruses inhabiting *Gremmeniella abietina* in Finland

Jarkko Hantula[1)], Tero T. Tuomivirta[1)], Stephane Vervuurt[1)], Juha Kaitera[1)] and Antti Uotila[2)]
[1)]Finnish Forest Research Institute; [2)]University of Helsinki

Scleroderris canker, caused by *Gremmeniella abietina*, is a major disease on pines and other conifers in northern hemisphere. *Gremmeniella abietina* type A and type B both host a divergent virus community: totiviruses and mitoviruses occur in both types, and partitiviruses in type A. Also viruses with very large dsRNA genomes (ca. 10 kb) were observed in both types. Although largely similar virus families occur in both types of *G. abietina*, the viruses are not closely related, and therefore not able to move between the types of *G. abietina*.

In a population genetic analysis the mitoviruses of *G. abietina* type A seemed to be genetically highly variable. In the analysis of molecular variance no genetic differentiation was observed between populations separated georgaphically by ca 200 km. Neither was there any differentiation between populations collected in two successive years.

The virus with a large genome in type B seemed to be distantly related to Endornaviruses of plants and oomycetes, but clearly separate. However, there was a high similarity to a short virus sequence available from *Discula destructiva*. This suggests that a unique undescribed virus family occurs in fungi.

Spatial and temporal variation in the occurrence of *Gremmeniella abietina* in Finland

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The aim of the study was to describe and analyze the spatial and temporal variation in the occurrence of the disease caused by *Gremmeniella abietina* in Scots pine stands in Finland. The study utilized data from forest health monitoring plots (Forest Focus Level I plots) (years 1985-2005) and temporal plots of the 8th and 9th National Forest Inventories. The purpose was also to demonstrate some possibilities of utilize such monitoring data for research purposes.

The National Forest Inventories showed that the disease was far more common in southern than in the northern parts of the country. The disease was also clearly spatially clustered. The proportion of diseased stands decreased between the 8th NFI (1986-1994) and 9th NFI (1996-2003). The change in the diseased area was statistically very significant, except in the most severe disease class ("complete damage").

The forest health monitoring revealed considerable changes in the occurrence of the biotic and abiotic damage over the years. Level I data can provide time series of the most important causes of damage. A heavy outbreak of new *Gremmeniella* was observed in 1988-89, and smaller peaks in 1997 and 2001. Apart from competition, *Gremmeniella* was among the most important identified factors that had increased needle loss (defoliation) in Scots pine.

Coarse temporal and spatial distributions of the most common causes of damage were also obtained using the level I data. The usability of various datasets were also compared with each other. It was evident, that the *Gremmeniella* epidemics in 2001, for instance, was partly missed by the NFI survey.

The relationships between the temporal and spatial patterns of the disease and the most important stand and environmental factors were analyzed using several approaches: classification and regression trees (in exploratory data analysis), hierarchical logistic regression models and GIS. It proved very difficult to model the changes in the disease occurrence in this data. Most of the weather variables were significant at least once, but the estimates of the effects were not coherent in different years. Despite this, some key stand and weather variables could be identified in the mixed model analysis. These included site fertility, stand density, temperature of the preceding winter and current early summer and the effective temperature sum.

Resistance differences between pine provenances against shoot and needle cast diseases

Martti Vuorinen
Finnish Forest Research Institute, Suonenjoki Research Unit

Nine pine (*Pinus sylvestris* L) provenances, two from Estonia and seven from Finland representing an area of about 1200 kilometers in south-north latitude were growing in three growing sites, two in Finland and one in Estonia. The conditions between the growing sites differed in temperature and in the length of growing season and also the day length during growing season. Generally all the pine provenances succeeded best in Suonenjoki research site, which locates almost as fast from the most southern and most northern provenances. Damages caused by Scleroderris canker were most severe in the most northern site and almost all of the five southern provenances destroyed. All together three different needle cast diseases occurred in provenance trial and the occurrence varied between sites: pine needle cast caused by *Lophodermium seditiosum* was most common in Estonia and only light infections could find in other growing sites. There were more infections in southern provenances than northern one. Grey needle cast caused by *Lophodermella sulcigena* was most common in northern site, Rovaniemi and slight also in Suonenjoki, but there was no differencies between provenances. *Lophodermella conjuncta* caused an epidemic in Suonenjoki and the first infections was found in local or more northern provenances, later on all the provenances were infected.

Session 2: Biological control of hardwood sprouting

Comparison of different *Chondrostereum purpureum* isolates to inhibition of sprouting of birch

Henna Vartiamäki[1)], Antti Uotila[2)], Jarkko Hantula[1)] and Pekka Maijala[2)]
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Sprouting of broad-leaved trees causes problems e.g. in young conifer stands and under power transmission lines. In these areas the most common method to avoid sprouting is mechanical cutting, which has to be performed at certain intervals to be effective. Researchers in the Netherlands and in Canada have demonstrated the potential of a white rot fungus, *Chondrostereum purpureum* (Pers. ex. Fr.) Pouzar, to successfully control stump sprouting of many broad-leaved trees. The aim of our study was to test the efficiency of different *C. purpureum* isolates as a biocontrol agent to control stump sprouting in Finland and to find out if the activity of several secreted hydrolytic and oxidative enzymes and growth ability on birch wood chips correlated with ability to prevent sprouting.

Enzymatic activity and growth ability on wood chips of 21 isolates of *C. purpureum* were tested in the laboratory. Based on the laboratory tests we chose four strains with high enzymatic activity and growth rate and four strains with low activities and poor growth rate for field experiment. In the middle of June 2006 birches were manually cut using a brush saw and the entire surface of the cut stems was immediately treated with one of the eight selected isolated. Control stumps were cut but treated with the blank inoculum or left untreated.

First assessment in the field was carried out 14 weeks after treatments. There were clear differences in the ability of different isolates of *C. purpureum* to prevent sprouting. Therefore it is worth to try to find more aggressive isolates in the future for biocontrol purposes. However, differences appeared in the field seemed not to correlate with high or low enzymatic activity on agar plates or growth rate in laboratory tests. Other characteristics which could affect to ability to prevent sprouting should try to find in the future.

<u>Questions:</u>

Marina Niemi: Have you noticed any effect of the treatment season?
Answer: Yes, there was a clear effect of the season, as the treatments in late spring – early summer were always more effective that during other time of a year.

Rimvys Vasaitis: What was the origin of your isolates of the fungus?
Answer: The used isolates were diploid strains derived from the fungal sporocarps.

Chondrostereum purpureum **as a biocontrol agent against undesired sprouting of deciduous trees**

Remigijus Bakys, Rimvydas Vasaitis, and Jan Stenlid.
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The objectives were to investigate: 1) relative pathogenicity of different *Chondrostereum purpureum* strains to a given tree species; 2) relative susceptibility of different tree species to a given strain of the fungus; 3) impact of felling season and size of felled trees to sprouting energy.
In the greenhouse, one *Bjerkandera adusta*, one *Cerrena unicolor*, one *Peniophora cinerea* and eight *Condrostereum purpureum* strains were tested for pathogenicity against five tree species: *Betula pendula*, *Alnus incana*, *Quercus robur*, *Fraxinus excelsior* and *Salix sp.* Pieces of wood 1×1×5 mm in size, autoclaved and pre-colonised with respective *Chondrostereum purpureum* strain, were used as an inocula. They were attached with a tape to a 1×5 mm size wound made at the base of a tree. For each species, 240 one-two year-old plants (for *Salix sp.*, single clone cuttings) were tested (20 plants per strain, plus 20 controls of sterile wood, 1200 in total). The experiment was arranged following randomized block design. From dead trees, the fungus was reisolated. The results showed pronounced differences both in pathogenicity of different strains, and in susceptibility of different tree species.
In the field, mycelial suspensions of *Bjerkandera adusta* and three *Chondrostereum purpureum* strains were applied to freshly cut stumps of 10-30 year-old *Betula pendula* during four months: beginning of June, July, August and beginning of November. A total of 3600 stumps were treated (including pure water controls), which were organized in repeated blocks. Measurement of stumps will and control effectiveness is not finished yet. However, preliminary results show that the control of sprouting by applying to stumps *Chondrostereum purpureum* is possible in practice and effectiveness of the same strains in greenhouse and field do correlate.

<u>Questions:</u>

Iben M. Thomsen: In what season of the year the four field experiments have been carried out?
Answer: Four field experiments have been carried out in very beginning of June, middle of July, second half of August and beginning of November.

Iben M. Thomsen: Does different season of felling and application of the fungus have any impact to birches ability to resprout?
Answer: The season of the year has an impact on the success of sprouting control. *Chondrostereum purpureum*, applied in the autumn had litle effect to control birch resprouting. However, so far we do not have data about significant difference of resprout in the fields, performed in the begining and the end of summer.

Henna Vartiamäki: How do you prepare inoculum for your experiments in the field?
Fungal mycelium was placed in flasks with liquid media and left for further growth for two weeks in the dark under room temperature. Consequently mycelium was separated from liquid media, placed to sterile and distills water and fragmented.

Fred Asiegbu: How can *Chondrostereum purpureum* kill the sprouts so fast?
Answer: Along with local decay, *Chondrostereum purpureum* carries fungal toxins in the wood tissues that causes leaf silvering and are able quickly kill the sprouts.

Genetic variation of *Chondrostereum purpureum* in Finland and Lithuania

Henna Vartiamäki[1)], Antti Uotila[2)], Rimvydas Vasaitis[3)] and Jarkko Hantula[1)]
[1)] Finnish Forest Research Institute; [2)] University of Helsinki; [3)] Swedish University of Agricultural Sciences

Chondrostereum purpureum (Pers. ex Fr.) Pouzar is a basidiomycetous white rot fungus commonly found on many deciduous trees, shrubs and fruit trees within the temperate zones world widely. *C. purpureum* has been shown to be a promising bioherbicide for controlling stump sprouting of *Prunus serotina* Erhr. in the Netherlands and of various broadleaf forest tree species in Canada. Sprouting of fast growing hardwood trees causes problems in many places such as young conifer plantations, under power transmission lines and roadsides.

Effectiveness and possible usage of native *C. purpureum* strains have been tested during the last few years in Finland. However, before large scale use we must know genetic diversity and presence of biotypes within species to avoid possible undesired surprises due to treatment.

We analyzed genetic variation of *C. purpureum* in Finland and Lithuania using random amplified micro satellite markers. The results showed high genetic diversity among the populations of this fungus, but almost a complete lack of local differentiation. Our results indicate that genetic gene flow occurs naturally throughout Finland and Lithuania. Therefore distributing any local genotype of this fungus as a biocontrol agent should not lead to introduction of novel genes or genotypes. The use of any native genotype as effective biocontrol agent should therefore be safe.

Questions

Jan Stenlid: There are two commercial products for inhibition of regrowth on cut stumps of deciduous trees in Canada? Why can not we use those already existing products for example in Finland? Do isolates collected from Canada and Europe differ from each other?
Answer: Using those already existing Canadian commercial product for example in Finland would be questionable due to well-known risks of introducing potentially pathogenic exotic fungi to new environments, even if they belonged to the same species. Compared to biological control treatment done with non-indigenous strain of the fungus, treatment with native strain is always less risky. Researchers in Canada have demonstrated that there are at least some differences in mitochondrial DNA of Chondrostereum purpureum collected from Canada and from Europe.

Märt Hanso: *Chondrostereum purpureum* is also known as a pathogen of fruit trees and ornamental trees causing silver leaf disease. Is it possible that C. purpureum will spread from the treated area to fruit trees?
Answer: We must remember that C. purpureum is very common fungus in nature. There are huge amount of naturally occurring spores in the air all the time. Of course it can be possible that spore density is getting a little bit higher close to the treated area. Therefore we should not do the C. purpureum treatment for example next to area of commercial fruit growing. Researchers in the Netherlands have recommended leaving 500 m safety zone between a control area and an area of commercial fruit growing.

Rimvys Vasaitis: Do you have any experience in using Dutch commercially available *Chondrostereum purpureum* BIOCON strain?
Answer: Personally, I have no experience, but Antti Uotila has tested it before my starting of work, and the results were intermediate as compared with the local strains.

Session 3. Decay and rot fungi

Genome mapping in the basidiomycete *Heterobasidion annosum* s. l.

Åke Olson[1)], Mårten Lind[1)], Kerstin Dalman[1)], Bo Karlsson[2)] and Jan Stenlid[1)]
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The bases for genetic linkage mapping and possible application in identification of tractable quantitative traits were demonstrated. Quantitative trait loci (QTL) for virulence on one-year-old *Pinus sylvestris* and two-year-old *Picea abies* seedlings were identified and positioned on a genetic linkage map of *Heterobasidion annosum sensu lato* (s.l.). The virulence among 102 progeny isolates was analysed using two measurements: lesion length around and fungal growth in sapwood from a cambial infection site. In general we found negative virulence effects of hybridisation. On *P. abies*, both measurements identified several partially overlapping QTLs on linkage group (LG) 15 of significant logarithm of odds (LOD) values ranging from 2.77 to 3.85. On *P. sylvestris,* the lesion length measurement also identified a QTL (LOD 3.09) on LG 15. Moreover, QTLs on two separate smaller LGs, with peak LOD values of 3.24 and 4.58 were identified for fungal sapwood growth and lesion lengths respectively. The QTL probably represent loci important for specific as well as general aspects of virulence on *P. sylvestris* and *P. abies*. This is the first report of QTL for virulence located in a genetic linkage map of the *Heterobasidion spp.* genome. The mapping of virulence QTLs forms the basis for positional cloning and identification of the corresponding virulence genes, which may give answers to the so far largely unknown question of which factors might be involved in the infection process of *H. annosum s.l.*

Sequencing the genome of the forest pathogen *Heterobasidion annosum*

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Heterobasidion annosum causes a devastating root rot in conifer plantations and natural forests throughout the northern hemisphere. In a collaboration with the Joint Genome Institute, the genome of *H. annosum* will be the first plant pathogenic homobasidiomycete to be sequenced allowing for new insights into plant-microbe interactions. Comparisons with plant pathogens with a gradient of taxonomic relatedness to *H. annosum* will help understanding the evolution of pathogenicity factors. Response of the model tree *Populus* to various types of trophic interactions can be studied including rust pathogen fungi and mycorrhizal mutualists. Furthermore, comparisons with the model white rotter *Phanerochaete crysosporium,* will deepening our understanding of wood degradation including ligninolytic and polysaccharide degradation pathways and several bioremediation applications. Moreover, this project will also gain insights into fungal evolutionary history and biology including development, non-self recognition, mating, and secondary metabolism.

Using immunolabelling and confocal microscopy for *in vivo* differentiation of the hyphae of *Phlebiopsis gigantea* from *Heterobasidion annosum* at barrage zones

Asiegbu, FO
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The saprophytic fungus *Phlebiopsis gigantea* has for several years been used as a biocontrol agent against the conifer pathogen (*Heterobasidion annosum*). However, very little is known about the mechanism behind *P. gigantea* mode of action. At cellular level, there have been suggestions that hyphal interference may be involved during the combative interaction. But distinguishing the hyphae of these two fungi within wood tissues poses a major challenge. The present study explored the feasibility of using a combined dual immunofluorescence labeling and confocal microscopy for detection of the two species *in vivo*. Over 48 monoclonal antibodies were screened using ELISA (Enzyme Linked Immunosorbent Assay) for their ability to specifically recognize hyphae of either *P. gigantea* or *H. annosum*. Two monoclonal antibodies met this criteria and were further investigated. The result revealed that with the aid of the dual immunofluoresence labeling technique it is possible to distinguish the hyphae of the two fungi within the barrage zone. Furthermore, at present, it is not clear what the antibody binds to, identifying the protein which the antibody binds to will hopefully assist studies on protein tagging and further exploitation of this technology in environmental monitoring.

Urea protection against *Picea abies* butt rot: results of a 15 year experiment

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In this study, we evaluate the long term effects of urea treatment of newly cut stumps against butt rot caused by *Heterobasidion annosum* in *Picea abies* stands. After the first thinning, the stumps of 16 experimental plots of *P. abies* in southern Sweden were: untreated thus subjected to natural infection, urea treated, half of the stumps urea treated and half artificially infected with *H. annosum* conidia (50% Urea + 50% Conidia) and artificially infected with *H. annosum* conidia (100 % conidia). After 15 years the incidence of butt rot was observed by extracting a bore core 20 cm above ground from all stems. Cores from rot trees were stored in sterile bottles, and observed under a dissecting microscope for *H. annosum* conidiophore presence. Urea treated stands showed significantly lower butt rot incidences (2%) than the other treatments, which were of 33%, 58% and 40% respectively for natural infection, 100% conidia, 50% Urea + 50% Conidia treatments. *H. annosum* was isolated from the 18 % of the cores derived from rotted trees, suggesting that it had caused the majority of rot. Urea treatment seems to be a reliable control method against *H. annosum* butt rot.

Do bark and wood-boring insects in mechanically created high stumps of Norway spruce facilitate the establishment of rot fungi

Ylva Persson and Jan Stenlid
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Saproxylic beetles are dependent on dead and dying wood. In Sweden high stumps are used as a nature care method to create dead and dying wood for the saproxylic beetles in the silvicultural forest.

Bark and wood boring insects are likely to habitat high stumps of Norway spruce (*Picea abies* L. (Karst.)). The role as a vector of fungal diseases and the fungal species composition in four different age classes of high stumps was investigated.

Material was collected as mycelia- wood sample and in/on insects found in bark sample in the different high stump age categories. Molecular identification using Polymerase Chain Reaction (PCR), DNA-sequensing, Terminal-Restriction Fragment Length Polymorphism (T-RLFP) and cloning of DNA fragments followed by sequencing were used for fungal species identification.

The sites had a majority of fungal species that were connected with the insect species except the site that were newly clear cut with no sign of insect activity. All the fungal species that occurred in the high sumps can be found both as mycelia, in the wood and on the insects that lives under the bark.

The result indicates that the insect facilitate the establishment of rot fungi cannot be neglected

Session 4. Epidemic diseases

Ash dieback in Sweden since 2002

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Shoot dieback affects common ash (*Fraxinus excelsior* L.) in the whole area of natural distribution in Sweden. The frequency of damages is high, why it is reasons to call it ash decline. This ash decline was known only in a few places in 2002, indicating a rapid and recent increase of an alarming emerging disease. In the summer 2004 decline and death of ash was reported widely in Sweden and in 2005 it increased further. So far no decrease is evident. The same type of disease was known in Lithuania and Poland for about ten years before we observed it in Sweden.

Detailed investigations of the disease symptoms showed leaf death, shoot dieback, branch and stem bark necrosis as well as canker formation ultimately leading to tree death generally over the whole area where ash is growing. In the spring shoot death is prominent and it may spread further the same season. Later in the summer new symptoms appear on leaves and branchlets. Pilot investigations in early September 2005 of these recent damages revealed one fungal species isolated in 90 % of the samples. The same high frequency was revealed from active stem cankers. The same fungus has also been consistently isolated from different types of wounds. The pathogenicity of the fungus has been tested in experiments both in greenhouse and outdoors and it was shown that it is pathogenic to ash. In 2006 Kowalski described a new fungal species associated with dieback of ash, *Chalara fraxinea* sp. nov. It is the same fungus as we have isolated and which we have found to be pathogenic. Still the infection biology and environmental conditions that promote the disease development are unknown.

Forest sanitary problems in the period of 1996-2006 in Lithuania

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Lithuania has over 2 million hectares of forest and it covers 32,5% of land. Approximately half of forests are private. Prevailing tree species are Scotch pine (35.8% of all forests area), Norway spruce (21.4%) and Silver birch (21.1%).

Ash decline (ash stands 2.5% of all forests area)

Ash decline is the biggest problem in Lithuania nowadays. It started in 1996 in central part of Lithuania (almost no ash stands in southern part) and was spreading to the north. It could possibly indicate that "disease" came from the south. Peak was reached in 2001 when more than 15 000 ha of ash stands (65% of all state owned ash stands) in state forests were recorded as damaged. There were about 9 000 ha of declining ash stands in state forests in year 2006, but area decreasing only because a lot of ash stands are clearcutted. You can barely find healthy looking ash stand in state or private forest in Lithuania today.

Primary causes for decline are still unknown. Different scientist have different opinions due to subject they are focused to. Some say it is change of climate with severe droughts, ground water level changes, high summer and low winter temperatures. Other claim it is related with ozone holes in the sky or due to game animal browsing (there were a lot of roe deers in 1970-1985) or bark beetle like *Hylesinus fraxini* activities or even radiation due to collapse of Chernobyl nuclear plant. Of course forest pathologists have their own opinion – some fungi in tree crowns, stem or roots is killing ash.

Something definitely happened. Ash stands of different age are dying. Young (less than 50 years old) stands, according to monitoring (held by Forest Sanitary Protection Service) data, degrade quicker (46% trees dead in 2006) than old (more than 50 years old) stands do (35% trees dead in 2006). Maybe it is because older trees have more and bigger roots/crown and it takes more time for fungi to rotten/kill them. In 2006 the biggest amount of dead trees in monitoring plots was recorded on wet (45% dead) or temporary wet sites (42% dead) and only 33%(dead) on sites with normal irrigation. Approximately 8-10% of ash trees are dead every year (monitoring started in 2002). Usually trees with 50% and bigger defoliation will be dead on next year.

Oak decline (oak stands 2.0% of all forests area)

There are 19 000 ha of oak stands in state forest in Lithuania. Oak decline started in 2004, but reached maximum in 2005-2006. During these three years about 16 000 ha of oak stands in state forests were more or less damaged. There were severe drought and high temperatures in 2002, massive outbreak (7896 ha) of green oak moth *Tortrix viridana* in 2003 sequencing oak mildew *Microsphaera alphitoides* and late spring frosts in 2005. That were the main reasons why oak decline started. Now it is over. Trees with 30% or less defoliation restored their crowns. Most of trees with 50% and more defoliation became dead. It looks like that older trees were more vulnerable for decline.

Birch decline

There are 180 000 ha of birch stands in state forest in Lithuania. Birch decline started in 2003 and peak was in 2004, but it was very small and local in comparison with ash or oak declines. Only 500 ha of birch stands were affected. The main reason was severe droughts in 2002 and birch leaf-roller beetle *Deporaus betulae* (983 ha) in 2003. Due to drought leaves got yellow and were lost already in the end of July or in August. Stands in forest edges near irrigation engineering trenches were mainly affected. Lose of leaves in the top of the crown was the main attribute of birch decline. Wood borer insects and fungi accompanied this process. Birch wood rottens very fast and trees with 50% or bigger defoliation had almost all stem wood brown by the end of summer.

Root rot of coniferous *Heterobasidion* sp.

Is the biggest problem in coniferous stands: both spruce and pine. 2869 ha of pine stands were dead cause of *Heterobasidion* sp. infections in 1996 in state forest in Lithuania. In successive years damaged stands fluctuated between 1664 and 809 ha depending on climate conditions. Real numbers of damaged stands are much higher. State forest enterprises show only root rot damage in pine stands and nothing in spruce ones. Besides, numbers of damaged pine stands are reduced because some of stands are wrongly evaluated as bark beetle killed stands. The main reason for root rot damage decrease after year 1996 was restitution of private property. The largest areas affected by root rot are in pine stands planted in former agriculture land in 1950-1960 (now mainly private forests). There will be more problem with root rot in the future because now a lot of pine and spruce are planted in former agriculture lands again.
Root rot in spruce stumps in clearcuts of mature spruce stands was evaluated in 1999-2001. Rotten spruce stumps were counted in 1243 clearcuts in nine state forest enterprises in western part of Lithuania. In average 24.8% of spruce stumps had visual rots.

Needle-cast of pine *Lophodermium seditiosum*

Biggest outbreaks were in year 2001, 2004 and 2005 – 4016 ha, 1075 ha and 879 ha of pine stands were damaged respectively. 1-5 years old forest plantations and natural regenerated pine stands were affected. The lose of needle has influence on pine health and on some sites damage of *Armillaria* sp. and *Heterobasidion sp.* occurred more intense. Chemical treatment is applied only in forest nurseries.

Dieback of conifers *Gremmeniella abietina*

Two big outbreaks of *Gremmeniella abietina* occurred in 1997 and 1999 – 1907 ha and 1039 ha of pine stands were damaged respectively. The difference between these outbreaks was that only forest stands were affected that in 1997. And in 1999 more 1000 000 of pine seedlings in forest nurseries were heavily damaged.

Pine stem rusts *Cronartium flaccidum*

A peak of *Cronartium flaccidum* damage (975 ha and 900 ha respectively) was in 1997-1998. It was related with outbreaks of needle grazing insects such as *Diprion pini* and *Panolis flamea*. The fungi is not considered as very important for forestry. Selective sanitary cuttings are applied to remove damaged trees. Fire wood is very expensive nowadays, so trees with pine stump rust are cut down intensively.

Phellinus tremulae (aspen stands 3.3% of all forests area)

There are 30 000 ha of aspen stands in state forest in Lithuania. About 65% of all aspen stands are mature and overmature – more than 40 years old. 7000 ha of the stands are heavily infected by *Phellinus tremulae*. And the cause of this situation is that it is not very profitable (almost no market for aspen wood in Soviet Union times) to cut down aspen stands, especially overmature and rotten ones.

***Phytophthora inflata*, a new alien species in Finland**

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Phytophthora ramorum was detected by species specific PCR for the first time in Finland in spring 2004 on marketed plants of *Rhododendron* spp. originating in other EU member states. In August 2004 the pathogen was also found in one Finnish nursery on German *Rhododendron catawbiense* plants and on several Finnish *Rhododendron* spp. cultivars. Symptomatic seedlings having necrotic lesions on leaves and stems were collected from the nursery in 2004 and 2005. The PCR-based screenings of samples were done according the protocol of the European and Mediterranean Plant Protection Organization (EPPO) for regulated pests (EPPO 03/10563). The molecular detection was thus based on primers specific for *P. ramorum.* To be sure that the pathogen was in living state in the samples, isolations were also done by plating sections from the margin of infected and healthy tissue onto modified PARP-agar and 1% malt extract agar. These isolations yielded in addition to *P. ramorum* two other *Phytophthora* spp. and isolates having conidia typical for *Pestalotiopsis* sp. In addition to morphological measurements a partial beta-tubulin gene of each *Phytophthora* species was sequenced, and identical sequences were searched from the Gen Bank. Our *P. ramorum* isolates were identical to *P. ramorum* CBS 109279 and CBS 101326. *P. cactorum* isolates produced oogonia and papillate sporangia on agar cultures and had genetically 100% match with *P. cacrorum* CBS 10809. The other homothallic *Phytophthora* showed a stellate growth pattern with sparse aerial mycelium. Oogonia had both paragynous and amphigynous antheridia, and sporangia produced in soil extract water were ellipsoid in shape and semi-papillate. The beta-tubulin sequence of *P. inflata* IMI 342898in the GenBank, had 100 % match with our isolates. The IMI 342898 strain has been isolated from *Syringa* sp. in the UK in 1990. It is likely that this *P. inflata* has been spreading in Europe with the ornamental plant trade.
To fulfill Koch's postulates *Rhodenrdon* plants were inoculated with *P. inflata* or *P. ramorum* and the presence of the pathogen in inoculated plants was verified by isolations. Both *Phytophthora* species caused also necrotic lesions on *Alnus glutinosa*, *A. incana* , *Betula pendula* and *Vaccinium myrtillus*. *Pinus sylvestris* was shown to be resistant to both *Phytophthora* spp. whereas *Picea abies* was susceptible to *P. inflata*, but not *P. ramorum*.
In August 2004 healthy looking rhododendrons from the nursery, which had had problems with *P. ramorum*, were planted in a garden. In summer 2005 planted rhododendrons died and the plants were removed. One year after that, in summer 2006, four *Vaccinium angustifolium* seedlings were planted near the place the rhododendrons had been grown in 2004-2005. In summer 2007, after a heavy rain and thunder storm in June, brown lesions were born on leaves of *V. angustifolium* plants. Isolations from these lesions revealed *P. cactorum* and *P. inflata*. This unintended episode proved that these alien species are able to survive in Finnish nature, although in a laboratory experment *P. inflata* cultures died in tree days at -5°C- a temperature common in soil during winter.

Lilja, A., Rytkönen, A., Kokkola, M., Parikka, P. & Hantula, J. 2007. First report of *Phytophthora ramorum* and *P. inflata* in ornamental rhododendrons in Finland. Plant Disease 91(8): 1055.

Willow leaf rust (*Melampsora epitea*) in Estonian energy forest plantations.

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Willow leaf rust caused by different forms of *Melampsora epitea* is a major disease problem in willow plantations established for biomass production. Diseased plants produce less biomass and may become more susceptible to frost and other pests. The occurrence of the pathogen depends on several factors and the aim of the study was to assess the effect of some of them. The assessments were carried out by counting the number of rust uredinia per leaf area unit on the leaves collected from different plantations. The results of the field studies in Estonia showed a strong correlation between rust abundance and weather conditions. The development and propagation of the disease was favored by humid and cool weather whereas on years with hot and dry periods during the summer only very few rust damages were detected. In addition to weather conditions, fertilization increased significantly rust levels compared to control plants. However, the effect of nutrient supply on rust abundance was different among studied clones. Since fertilization increases significantly the biomass production it is essential to find clones on which the rust abundance is only slightly affected by changes in nutrient levels in soil. In future it is important to use these clones when establishing new plantations to diminish the losses caused by leaf rust fungus.

Oak decline in Lithuania

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Starting in 2003, the last and probably one of the most severe waves of oak decline caused serious sanitary problems in Lithuanian broadleaved forest ecosystems. In 2006, about 16,000 ha of oak stands were reported to be to greater or lesser extent damaged by an unknown disease; within this area, 30% of trees were regarded as declining or dead. Rates of the decline dropped in 2007, and some of the diseased trees have recovered.

Drought and lowering of a ground water level in 2002, severe autumn and spring frosts in 2003-2004, increase in herbivorous pest populations in 2002-2003, and secondary defoliation caused by powdery mildew could be the main reasons that induced the recent decline.

Priori to our study some pilot studies have been performed by a staff of Lithuanian Forest Sanitary Protection Service. Sixteen declining oaks were felled and their cross-sections (at stump level) were checked for the presence of tracheomycosis. Blocked water ducts in sapwood were found in sections of two oaks. Seven of the felled oaks were also investigated for a distribution of ophiostomoid fungi along the stems (later it was confirmed to be *Ophiostoma quercus*). It has been found that in most cases the fungus (or fungi?) is distributed all along the stems of diseased trees: from top branches to the stump level (moist chamber method was used to incubate sections from various parts of a stem).

Aims of the present study were: 1) to investigate fungal community in wood of the declining oaks; and 2) to check pathogenicity of the "prime suspect" *O. quercus* and of other most commonly isolated wood-inhabiting species (from the declining oaks). During spring-summer of 2006, 25 declining oak trees in three locations in diseased stands have been felled, and a total of 640 wood samples were taken from the stumps and branches (380 samples from 25 stumps and 260 samples from 75 branches). The isolation of fungi on Hagem agar media resulted in over 500 fungal strains representing 93 distinct operative taxonomic units (OTU's). 56 (60%) of those OTU's were identified at least to a genus level. All of the 25 sampled wood blocks were incubated in a moist chamber for a couple of weeks to check for a presence of reproductive organs of ophiostomoid fungi. Perithecia and coremia typical of *Ophiostoma* sp. were produced on almost every wood block. Isolation from those organs and molecular identification confirmed it was *O. quercus.*

In autumn 2006, a total of 210 acorns have been collected in the diseased oak stands and sowed in pots in a growth chamber. 99% of the acorns germinated. Another 10 acorns have been inoculated by one strain of isolated *O. quercus* just before planting; from this number, 7 acorns germinated and the seedlings look healthy so far.

In the beginning of September 2006, 9 strains of *O. quercus*, along with 7 strains of other most commonly isolated OTU's were used for inoculation of 215 eight-year-old oaks (13 replications were made in total). Similar experiment was performed in the beginning of

June 2007, where 207 eight-year-old oaks and 192 eight-month-old oak seedlings (the latter grown in pots in a growth chamber) were inoculated with mostly the same fungal strains. None of the 8-year-old oaks showed symptoms of the disease, and there is little evidence of pathogenic activity of the inoculated fungi in 8-month-old seedlings so far. Another growing season (or even longer time) is needed to gain more straightforward results. Non-destructive re-isolation of the inoculated fungi will be done in the nearest future.

Conclusions:

- Tracheomycosis of water ducts can be one of the reasons of the decline (at least for some oaks);
- Germinability of acorns from diseased stands is very good, seemingly it has not been affected by any disease;
- *O. quercus* is very common in wood (all along the stem) of declining oaks, yet it’s role in the decline process remains unclear;
- Preliminary results of the inoculation experiments suggest that pathogenicity of tested *O. quercus* and other strains is low (?) – more time is needed to gain straightforward results.

Session 5: Special issues on forest tree diseases

Some ongoing research and future research interests with spruce cone rusts

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Cone and seed pathogens reduce the amount and quality of annual seed crop. In years with excellent cone production the role of pathogens increases. The variables affecting epidemics are poorly studied and known, which complicates disease control. The most important cone pathogens are cone rusts, *Thekopsora areolata* and *Chrysomyxa pirolata*.

In a new project 'Possibilities for integrated control of cone and seed insects and diseases in Norway spruce' the frequency of cone rusts in cones collected from a number of seed orchards and seed tree stands in 2006, and the time of fruiting of cone rusts in case-orchards and stands during growth period in 2007 are investigated. In the future, the virulence of the rusts, especially of *T. areolata*, and the susceptibility of some potential and commonly in the ground vegetation growing plant species to *T. areolata*, will be investigated.

Based on the very preliminary sample collection and analysis in the case-orchards during the early 2007, both rusts occurred commonly in cones formed in 2006. *Thekopsora areolata*, however, occurred in almost all the sample cones. In cones formed in 2007, *T. areolata* was rare but *Chrysomyxa* spp. common in the case-orchards. The reasons for variation in epidemics of the cone rusts are discussed.

***Laetiporus sulphureus* s.l.: ecology and genetic diversity**

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The aims of this work were to check *Laetiporus sulphureus* s.l. phylogeny in different geographic areas of the world, to relate to obtained data to already existing information, and to compare *in vitro* growth of different phylogenetic groups (species). The materials included 130 *Laetiporus* individual isolates/fruitbodies from at least 20 host tree species collected in Europe (Sweden, Denmark, Lithuania, Latvia, Czech Republic, Spain), Canada, Uruguay, South Africa and Korea. The methods included both ITS sequencing and *in vitro* growth experiments.
Initially (apart from Korean sample), the analysis of the results of ITS sequencing has revealed four clusters. Cluster A included part of the samples from Europe, collected from *Quercus*, *Fraxinus*, *Salix*, and part of the samples from Uruguay, collected from *Eucalyptus* and *Prunus*. Cluster B included another part of isolates from Europe (only), collected from *Quercus*, *Salix*, *Fraxinus*, *Prunus*, *Pyrus*, *Populus*, *Juglans* and *Cladrastis*. Cluster C included two well supported subclusters, – one from Canada (on *Pseudotsuga*) and one from Europe (on *Picea*), seemingly found only on conifers. Clusters D and E represented respective two well defined groups, one from South Africa and one from Uruguay (another part), which in both geographic areas occurred exclusively on *Eucalyptus*.
When related to available GenBank data, Cluster A (deciduous, Europe & Uruguay) was joined by specimens found on *Quercus* and *Castanea* from Europe, and unknown host tree specimens from Spain, and North America, indicating possibly even wide geographic range. Cluster B (deciduous, Europe only) was joined by specimens found on *Salix*, *Prunus* and *Picea* (!) from Europe, indicating that this group might be not strictly adapted to deciduous trees. Conifer Cluster C was joined by another specimen collected from *Picea* in Switzerland. Cluster D (*Eucalyptus* S.Africa) and Cluster E (*Eucalyptus* Uruguay) has remained separate from each other and all the rest. Furthermore, our samples from Korea formed two another clusters, – Cluster F (collected from *Quercus*, *Carpinus*, *Celtis* & unident. Deciduous, and *Abies*), and Cluster G (collected from *Quercus*, *Carpinus*, *Castanea* and other deciduous trees). Those two clustered distinctly also from the rest.
Growth experiments with three groups (two strains from each) included Cluster A (Europe & Uruguay), Cluster B (Europe only) and Cluster E (Uruguay only), and were performed on seven different agar media, in three temperature regimes (20, 24 and 28 C). Linear growth rate and dry mass accumulation were more similar between the two representatives from each Cluster, than among diffrent Clusters, indicating that those might represent distinct biological entities.

<u>Questions</u>

Risto Kasanen: Do you think it would be worth checking several markers for phylogenetic analyses? ITS is a good marker, but in this case it is just single one.
Answer: Agree, that more markers would be a good idea, but in this case we have also measurements of growth, which in general support data from ITS markers.

Jarkko Hantula: One should be careful to imply separation of genus into the species based solely on ITS. We (Michael Muller) have some examples when some ascomycetes while having different ITS types in fact are the same species.
Answer: In this study we do not claim the division of *Laetiporus* into the separate biological species. We do not refer to clusters as species, but rather as to groups. And the overall aim was just to check genetic diversity based on the single ITS marker.

Märt Hanso: Could not it be a mistake in the GenBank when your Europen deciduous cluster is joined by the GenBank strain from spruce? Before in your data, all specimens collected from conifers were making a single well defined group.
Answer: It is unlikely to be a mistake. We already know, that not all *Laetiporus* individuals from conifers in Europe cluster into the single group. We have more samples which we are now proceeding. Therefore this German GenBank strain might in fact come from another group that is mixed with deciduous.

Wounds and decay on urban trees

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Trees play a central role in built-up urban environments (parks, streets, yards) in creating pleasant health-promoting neighbourhoods. Moreover, urban trees are associated with considerable financial aspects. Management costs can be considerable when operating amidst dense urban structures, where special skills and equipment are needed.

Since 1999 the parks in Helsinki have been undergoing an extensive restoration programme including urban tree inventories with risk assessments. There are about 150 000-200 000 park trees and over 25 000 street trees in the Helsinki city area. The most common urban tree species are Tilia spp., Betula spp., and Acer spp. Of park trees about 18% are Acer spp., 14% Tilia spp. and 11% Betula spp., and of street trees about 10%, 50% and 10%, respectively. The inventories have shown that a large number of old park and street trees are in a poor condition.

The present research project was established to improve the management and protection of old urban trees in the Helsinki City, and to help the authorities in timing the removals of trees in poor condition. The aim of the project is to increase our knowledge of the interaction between external injuries and the internal decay of the most common urban tree species in Finland. We also wanted to investigate which pathogen species are causing the severest problems to tree health in urban surroundings in Finland.

A total of 256 felled urban trees were examined during 2001-2003: 95 *Tilia* spp., 74 *Betula* spp., and 87 *Acer* spp. Most of the trees (73%) were located in the main parks and along the main streets in the downtown area of Helsinki City. The mean age of the trees was over 60 years, and the majority (64%) were old park trees. Poor condition and increasing risk of failure were the main reasons for felling in 82% of the cases. 33% of these trees were degenerated or dead, but the amenity value of 14 % of the risk trees was still high. Some characteristic profiles for potential failure were identified for each of the tree species studied: *Ganoderma lipsiense* in the butts and hollows in the stems of *Tilia* spp., weak fork formations together with *Rigidoporus populinus* on *Acer* spp., and degeneration together with decay in the stem on *Betula* spp.

Thirteen species or genera of commonly occurring decay fungi were identified on the basis of their fruiting bodies and pure cultures. The occurrence of the fungi was investigated in terms of the frequency, visibility, and as a potential cause for stem breakage. Most hazardous fungi caused extensive horizontal decay in the stem; such fungi were *Ganoderma lipsiense* on *Tilia* and *Acer*, *Phellinus igniarius* on *Acer*, *Inonotus obliquus* and *Cerrena unicolor* on *Betula*, and *Kretzschmaria deusta* on *Acer*, *Tilia* and *Betula*. A typical characteristic of *Rigidoporus populinus* was its frequent presence in weak fork formations of *Acer* trees. Agaric fungi (*Pholiota*, *Armillaria*, *Pleurotus*, *Hypholoma*) were frequently recorded but they were of minor importance from the point of view of tree breakage hazard.

Publications:

Terho, M & Hallaksela, A-M. 2005. Potential hazard characteristics of *Tilia*, *Betula*, and *Acer* trees removed in the Helsinki City Area during 2001-2003. Urban Forestry & Urban Greening 3:113-120.

Terho, M., Hantula, J., Hallaksela, A-M. Occurrence and decay patterns of common wood-decay fungi in hazardous trees felled in the Helsinki City. Forest Pathology. (in press).

Alterations of Scots pine growth and needle characteristics after abrupt winter onsets: a retrospective analyses using the needle trace method (NTM)

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The presentation belongs to the field of unpopular for a long time, but recently demanding larger attention field of forest pathology – abiotic damages/diseases.
Two years ago in Biri, Norway we introduced the first part of the diagnostic approach to a complicated case of natural disturbances in Scots pine (*Pinus sylvestris*) in south-eastern Estonia in 2002 by the involvement of needle trace method (NTM). Definitely, a series of unfavourable biotic, but mainly abiotic factors might have a role in the massive-scale stress and death of trees, incl. Scots pines in forests there.

In this investigation we have turned our attention to the influence of abrupt winter onsets to pines. The second task was to analyse, how could a series of extreme seasons namely summer drought, abrupt winter onset and cold first half of the winter alterate pine needle and growth characteristics. Most extreme seasons were selected out from the meteorological data inside the period 1866-2006 ("the long period") in different variants, concerning mean monthly temperatures:

Forty three Scots pine trees grown up in five different stands in south-eastern Estonia were analyzed, regarding the NTM protocols.

Comparing the reaction of trees after three variants of winter onsets, it was found that the most inferior to the Scots pine was the variant, when temperature drops down suddenly from August to October (and to November). It was concluded, that a bad winter onset alone could not be a killer, but might act as a serious stressor. Similar results were before obtained for the extremely droughty summers and cold winters, separately taken. It was found that unfavourable **series of seasons** acted as hard stressors and killers.

The most extreme series of seasons fell to the years **1939/1940** and **1941/1942**, representing definitely the record inside the long period (1866-2006. Nearly similar series of extreme seasons occurred in Estonia in **2002/2003**, affecting similarly as pine growth, as well as needle characteristics. Influence of the last series seemed to be even more severe, because there were predisposing events: the summer drought in 1999 and *Lophodermium* needle cast epidemic, which lasted 3 years (1999-2001).

Logically, the reaction of trees to the sample of three most extreme years with the series of bad seasons was more pronounced than to the sample of five years.

Session 6: Factors influencing the diseases

Role of secondary metabolites in chemical defense of poplars

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Several studies have suggested that phenolic secondary metabolites play an important role in the defense of aspens and poplars (*Populus* sp.) against insect herbivores. On the contrary, much less is known about the importance of phenolic compounds to pathogen resistance of these trees. This gap in our knowledge may reflect the great spatial and temporal variation and dynamics of pathogen infections, which complicates detection of chemical interactions with traditional analysis methods. New information about the importance of phenolics in resistance of poplars may, however, be gained from controlled experiments with genetically modified poplar lines showing altered phenolic profiles. Moreover, analysis of the complete set of small-molecule metabolites (including secondary metabolites, hormones, and metabolic intermediates), i.e., metabolic profiling, is expected to give us a more holistic picture of the metabolic status in different interactions between poplars and their natural enemies.

Impact of mycorrhizal inoculation on establishment and growth of conifer seedlings on abandoned farmland

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The aim of the study was to investigate whether inoculation of *Pinus sylvestris* L. and *Picea abies* (L.) Karst. seedlings with mycorrhizas of *Cenococcum geophilum* Fr., *Piceirhiza bicolorata* and *Hebeloma crustuliniforme* (Bull.) Quel. has any impact on: a) survival and growth of outplanted seedlings on abandoned farmland, and b) subsequent mycorrhizal community development. For inoculation, the root system of each plant was wrapped in a filter paper containing mycorrhizal mycelium, overlaid with damp peat-sand mixture and wrapped in a paper towel. In total, 8000 pine and 8000 spruce seedlings were planted on 4 ha of poor sandy soil in randomized blocks.
Already after the first year natural mycorrhizal infections prevailed in the inoculated root systems while introduced mycorrhizas colonised less than 30% of fine roots. The seedlings that had been pre-inoculated with *C. geophilum* and the *P. bicolorata* during the whole four-year assessment period showed significantly higher survival and growth as compared to controls indicating that inoculation effect persists for several years. Moreover, the independent colonisation of roots by *C. geophilum* and the *P. bicolorata* from natural sources was also observed. A diverse mycorrhizal community was detected over two growing seasons in all treatments, showing low impact of inoculation on subsequent fungal community development. A total of 19 additional ectomycorrhizal morphotypes was observed, which clustered into two well separated groups, according to host tree species (pine and spruce).
In conclusion, the results showed limited ability to increase substantially tree survival and growth, and to manipulate the mycorrhizal community even by extensive pre-inoculations, indicating that establishment of plants and fungal community formation in root systems is governed mainly by environmental factors.

<u>Questions:</u>

Iben Thomsen: What was the reason for seedling dieback in the plantation?
It seems that in many cases the environmental factors were responsible for dieback of seedlings. However, in some cases the pathogenic fungi (e.g. *Fusarium* spp., *Nectria* spp.) were also recovered from the roots of declining seedlings.

Jonas Oliva: What was the actual effect of inoculation?
In treatments of *Cenococcum geophilum* and *Piceirhiza bicolorata* survival and growth of plants was increased by 10-15% as compared to the control. In forestry such effect of mycorrhizal inoculation is apparently too low for application on a large scale. Nevertheless, this effect would be substantial for establishment of seedling on marginal habitats (sandy dunes, excavated gavel pits, etc.) where using routine planting practices survival of seedlings is frequently very low.

Vaidas Lygis: Is the same fungal strains were used for mycorrhizal inoculation?
Yes, all plants within respective treatment were inoculated with the same fungal strain.

Arja Lilja: Why the inoculation of seedlings was not carried out in forest nursery in order to increase the success of root mycorrhization before outplanting?
The aim of the study was to test whether inoculation of seedling roots at outplanting has any effect on seedling survival and growth. The inoculation of seedlings in the nursery is one among other inoculation methods available. However, inoculation in the nursery can also result in roots colonisation at higher extent

by indigenous nursery mycorrhizas like *Thelephora terrestris* which is know to have little beneficial effect on plant survival after outplanting.

Rimvys Vasaitis: What was the extent of roots colonization by inoculated mycorrhizas under the laboratory conditions?
More than 50% of fine roots were colonised by target mycorrhizal fungi under laboratory conditions.

Elm (*Ulmus glabra*) in Norway – a threathen species?

Halvor Solheim, Skog og landskap, Norway.

The first pandemic of the Dutch elm disease caused by *Ophiostoma ulmi* was not really serious in Norway. Only twice (1963 and 1972) a few trees was recorded and felled. From 1981, when the new species, *Ophiostoma novo-ulmi* reached Norway, it took only twelve years before the authorities decided to give up the sanitary program around the Oslo fiord area. Fourteen years later we investigated the situation for the elm trees (*Ulmus glabra*) and the Dutch elm disease in an area heavily infested in the early 1990ties, Lier valley. In 2006 around 4% of the trees were infested by *O. novo-ulmi*. In 2007 only a few trees were infested.. Preliminary results of the elm registration show that the volume of elm in Lier valley have increased since the sanitary program was shut down in 1993. The increment of the living trees and the re-growth of young trees compensate for the loss caused by Dutch elm disease.

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