Program and Abstracts of the SNS PATHCAR Nordic / Baltic Forest Pathology Meeting, 28th of September – 2nd of October 2009, Palanga, Lithuania

Monday, 28th of September

Arrival and accommodation at Hotel "Alka". Registration and dinner for earlier arrivals, snacks and refreshments on bus for travelers from Riga Airport

Tuesday, 29th of September

7:00 – 8:40. Breakfast and registration

8:40 – 9:00. Welcome and introduction: **Rimvys Vasaitis & Co**.

"HETEROBASIDION" session, part 1. Chair: **Jarkko Hantula**.

9:00 – 9:20. **Jan Stenlid**: "Can genome sequencing help counteract root rot?"

9:20 – 9:40. **Michael Müller**: "Response of wood decomposition by *Heterobasidion parviporum* to temperature changes and differentiation of subpopulations according to local climate".

9:40 – 10:00. **LiYing Wang**: "Lower limit of stump diameter to spread *Heterobasidion annosum*".

10:00 – 10:20. **Talis Gaitnieks**: "Occurence of *Heterobasidion* fruit bodies on *Picea abies* in Latvian forests".

10:20 – 11:00. Coffee/ tea break

"CHONDROSTEREUM" session. Chair: **Halldór Sverrisson**

11:00 – 11:20. **Jarkko Hantula**: "Potential of *Chondrostereum purpureum* as a sprout control of birches in Finland".

11:20 – 11:40. **Vaidotas Lygis**: "Fungi for biocontrol of *Robinia pseudoacacia*, an invasive tree species in Lithuanian coastal ecosystems".

11:40 – 12:00. **Natalija Arhipova**: "Rot-causing basidiomycetes in gray alder (*Alnus incana* (L.) Moench.) in Latvia".

12:00 – 12:20. **Remigijus Bakys**: "Effect of *Chondrostereum purpureum* treatment on sprouting of birch stumps: field experiments".

12:20 – 13:40. Lunch break

"NEEDLE AND SEEDLING" session. Chair: **Timo Kurkela**.

13:40 – 14:00. **Taina Pennanen**: "Ectomycorrhizal fungi on Norway spruce - a seedling growth rate perspective".

14:00 – 14:20. **Martti Vuorinen**: "Red band needle blight in Finland, current situation".

14:20 – 14:40. **Hanna Millberg**: "Fungal flora of Scots pine needles, variation with altitude and latitude".

14:40 – 15:00. **Raija-Liisa Petäistö**: "*Fusarium avenaceum* and *Picea abies* container seedlings/ light and winter storage conditions, preliminary results".

15:00 – 15:20. **Audrius Menkis**: "Establishment of ectomycorrhiza inoculated seedlings of *Pinus sylvestris* on coastal dunes following forest fire".

15:20 – 16:00. Coffee/ tea break

"RUST" session. Chair: **Iben Thomsen**.

16:00 – 16:20. **Pia Barklund**: "Resin top rust disease of Scots pine caused by *Cronartium flaccidum*/ *Peridermium pini* in northern Sweden".

16:20 – 16:40. **Halldór Sverrisson**: "The effect of poplar leaf rust on frost damage of hybrid poplars".

16:40 – 17:00. **Merje Toome**: "Some causes for increased leaf rust abundance in wastewater treated willow plantations".

17:00 – 17:20. **Timo Kurkela**: "Morphological differences in uredinial stage of aspen rust, *Melampsora populnea*".

17:20 – 18:30. Free time

18:30. Dinner

Wednesday, 30th of September

7:00 – 8:30. Breakfast

8:40 – 18:00. EXCURSION – 1 (program at the end of this document).

18:30. Dinner

Thursday, 1st of October

7:00 – 8:30. Breakfast

"FUNGI AND INSECTS" session. Chair: **Vaidas Lygis**.

8:40 – 9:00. **Valentyna Meshkova**: "Entomological problems for forest regeneration in clear-cuts and burned areas of Forest Steppe and Steppe of Ukraine".

9:00 – 9:20. **Kateryna Davydenko**: "Causes of damage to *Pinus sylvestris* L. plantations on old burnt forest area in Ukrainian Steppe".

9:20 – 9:40. **Rimvys Vasaitis**: "Population structure of *Cerrena unicolor*, wood-decay polypore symbiotic with the horntail *Tremex fuscicornis*".

9:40 – 10:00. **Olga Kukina** & **Yuriy Skrylnyk**: "Colonization of trees and coarse woody debris by xylophagous insects on post-fire clear-cuts".

10:00 – 10:20. **Ylva Persson**: "Fungi vectored by the bark beetle *Ips typographus* following hibernation under the bark of standing trees and in the forest litter".

10:20 – 11:00. Coffee/ tea break

"HETEROBASIDION" session, part 2. Chair: **Jan Stenlid**.

11:00 – 11:20. **Ari Hietala**: "Growth strategy of *Heterobasidion parviporum* within stems of Norway spruce".

11:20 – 11:40. **Kristine Kenigsvalde**: "A rapid method for testing growth rate of *Phlebiopsis gigantea* isolates".

11:40 – 12:00. **Anthony Mgbeahurike**: "Differential screening of *Phlebiopsis gigantea* isolates for traits associated with biocontrol of the conifer pathogen *Heterobasidion annosum*".

12:00 – 12:20. **Jonàs Oliva**: "Recent advances on the control, epidemiology and detection of *Heterobasidion annosum* in *Picea abies* in Scandinavia".

12:20 – 13:40. Lunch break

"ASH DIEBACK" session. Chair: **Talis Gaitnieks**.

13:40 – 14:00. **Iben Thomsen**: "Impact of ash decline in Denmark".

14:00 – 14:20. **Lea Vig McKinney:** "*Fraxinus excelsior* shows genetic differences in susceptibility towards *Chalara fraxinea*, novel ash dieback".

14:20 – 14:40. Halvor Solheim: ”Ash dieback in Norway”.

14:40 – 15:00. Stina Johansson: ”*Chalara fraxinea*, biology and genetics”.

15:00 – 15:20. **Arja Lilja**: “*Phytophthora cactorum* and some recently found, non-native pathogens in Finland”.

15:20 – 16:00. Coffee/ tea break

“FUNGAL DIVERSITY” session. Chair: **Halvor Solheim**.

16:00 – 16:20. **Roger Finlay**: “Vertical structure of ectomycorrhizal species composition in relation to soil phosphorus sources”.

16:20 – 16:40. **Indrek Sell**: ”The ecology of *Peniophora junipericola* (Basidiomycota, Russulales)”.

16:40 – 17:00. **Lelde Grantina**: “Particular characteristics of soil microbial communities in forest stands infected with *Heterobasidion parviporum* and *Armillaria* spp.”

17:00 – 17:20. **Emad Jaber**: ”Molecular factors regulating Scots pine Antimicrobial peptides (SP-AMP) gene expression”.

17:20 – 17:40. **Ieva Vasiliauskaite**: ”Diversity of wood-inhabiting fungi in stems and roots of mountain pine (*Pinus mugo* Turra.) with special emphasis on root rot-causing basidiomycetes”.

19:00. Dinner

Friday, 2nd of October

7:00 – 8:30. Breakfast

8:40 – 9:00. Business meeting.

9:00 – 10:00. POSTER SESSION (presentations below).

10:00 – 12:00. EXCURSION – 2 (program at the end of this document).

12:00 – 13:00. Lunch

13:10. Departure of bus to Riga Airport.

POSTER SESSION, presentations

1. Janis Donis: “A long-ago promised poster”.

2. Märt Hanso & **Rein Drenkhan**: ”Multiproxial approach to the epidemiology of *Lophodermium* needle cast of Scots pine (*Pinus sylvestris* L.) in Estonia”.

3. Tuula Piri: ”Control of *Heterobasidion* root rot by stump removal - the ongoing field trials in Finland”.

4. Tuula Piri: ”Spread of *Heterobasidion parviporum* in an uneven-aged managed Norway spruce stand”.

5. Sannakajsa Nylund: ”Ectomycorrhizal (ECM) community structure: the role of host tree genotype”.

6. Heikki Nuorteva: ”*Gremmeniella abietina* and artificial pruning induce long-term alterations in foliar chemistry of Scots pine”.

7. Eeva Vainio: ”*Heterobasidion* spp. fungi host a diverse pool of partitiviruses that show evidence of horizontal transmission between species”.

8. Anna Rytkönen: ”PCR-DGGE method for detection and identification of *Phytophthora* species from plant material” (poster).

9. Elna Stenström: “*Gremmeniella* infections on seedlings after replanting severely infected pine forest”.

10. Berit Samils: ”Evidence of asexual overwintering of the willow leaf rust fungus *Melampsora larici-epitea*”.

11. Johanna Witzell: “Endophytic fungi in elms – characterization and interactions with DED fungi”

EXCURSION – 1

(program by **Vaidotas Lygis** & **Ieva Vasiliauskaite**)

Venue: Curonian Spit National Park.
Aim: to demonstrate experimental sites established within EU Marie Curie LITCOAST project (coordinator: R. Vasaitis, Sweden – Lithuania) and scenic landscapes of Baltic coastal ecosystems.

8:40. Bus from "Alka" hotel.

1st stop (at about 10:00-10:30, for ~45-60 min): a post-fire site in a 100-year-old mountain pine (*Pinus mugo* Turra.) forest burned down in May 2006 by a severe forest fire in Smiltyne Forest District (Kuršių Nerija National Park). Several experimental sites (mostly – investigated by the EC-funded LITCOAST project initiative) will be demonstrated:

a) experimental Scots pine plantation established one year after the fire with seedlings artificially inoculated with ectomycorrhizal fungi - a short presentation by Audrius Menkis;
b) a post-fire site: investigation of the diversity of wood-inhabiting fungi; survey of *Heterobasidion* disease centres, experimental plantations on the identified active disease centres – a short presentation by Vaidotas Lygis and Ieva Vasiliauskaite;
c) control of invasive tree species - black locust (*Robinia pseudoacacia* L.) with *Chondrostereum purpureum* (Pers.) Pouzar – an experiment started in July, 2009: a short presentation by Vaidotas Lygis and Remigijus Bakys.
d) mountain pine stands suffering from a disease caused by *Rhizina undulata* Fr. at the edge of the burned site.

2nd stop (for ~15 min): a special demonstration site for tourists – a burned mountain pine stand (left as it is).
3rd stop (for ~45 min): a resort town of Juodkrante – visiting the Witches' Hill, a nice exposition of wooden carvings and old-growth forest of Scots pine on sandy dunes.
4th stop (for ~45-60 min): nesting site of the great cormorant and grey heron near Juodkrante: damage caused by birds to the old-growth forest. Lunch (snacks) & coffee in the open air (in the bus?).
5th stop (for ~45-60 min): visit to the Nagliai Nature Reserve – a nice and impressive path through the Lithuanian "Sahara desert" to the Curonian lagoon.
6th stop (for ~30-45 min): stabilization of the moving sands by *Rosa rugosa* Thunb. at Baltic Sea coast near a resort village of Preila. And the beach (:
7th stop (for ~15-20 min): old *Heterobasidion* disease centre in a mountain pine stand, replanted by Scots pine and birch in 1982 near a resort town of Nida: an example of success. Newly occurring infection foci in an adjacent stand: a clearcut and further expansion of the root rot.
8th stop (for ~20 min): declining stands of black alder (*Alnus glutinosa* (L.) Gaertn.) close to the Russian (Kaliningrad) border.

9th stop (for ~20-30 min): stand of mountain pine severely damaged by the root rot near Nida – efforts to get the forest back. Old *Heterobasidion* disease centres in a mountain pine stand, replanted by Scots pine and birch in 1991: an example of sad story.
10th stop (for ~60 min): coffee and snacks on the Parnidis Dune. There is a solar clock and a most famous sight-seeing place in vicinity of Nida town on the top of the Dune. Here we'll make a group photo (?).
Way back for a dinner to Palanga. On our way we'll show you (pass across) Nida resort town if we get a permission for our big bus.
11th stop (final): arrival to our hotel in Palanga.

EXCURSION – 2

(program by **Rimvys Vasaitis**)

Venue: Palanga and surroundings.
Aim: LITCOAST project experimental sites and Palanga Amber Museum.

8:40. Bus from "Alka" hotel.

1. Plantations of deciduous trees in *Heterobasidion* disease centres.
2. Coastal Scots pine ecosystem: a setting for studies on fungi in soil and needles (Roger Finlay & Jan Stenlid)
3. Palanga Amber Museum.

Abstracts of the SNS PATHCAR Nordic / Baltic Forest Pathology Meeting, 28th of September – 2nd of October 2009, Palanga, Lithuania

Can genome sequencing help counteract root rot?

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Annosum root rot is one of the most devastating diseases in conifer forests. It is caused by the necrotrophic basidiomycete *Heterobasidion annosum s.l.* consisting of a species complex with partly overlapping geographic distributions and host ranges. Recently, the genome of *H. annosum* has been sequenced and annotated. The analysis of the gene content showed presence of the basic gene sets necessary for wood decomposition but revealed also several protein domain families with significant expansion in *H. annosum* in comparison with other basidiomycetes and plant pathogens that include candidates responsible for the specific life style of the fungus. We have constructed a genetic linkage map, recently transferred to the physical gene map, and identified several QTLs that are associated with e.g. pathogenicity, growth rate and fungal interactions, these also give candidate genes for host interactions. Transcriptome analysis indicate that coping with oxidative stress, producing secondary metabolites, degrading wood components and detoxifying host defence reactions are part of the arsenal activated in contact with living host tissue. Studies are on the way to silence or knock out candidate genes in the fungus to verify the importance of several of the indicated candidate genes. Transcriptome analysis of the host tree *Pinus sylvestris* indicate that response to pathogen presence is building up with time and includes cell death, induction of phenolpropanoid pathway and induction of antimicrobial proteins.

Response of wood decomposition by *Heterobasidion parviporum* to temperature changes and differentiation of subpopulations according to local climate

Michael M. Müller[1], Nicola La Porta[2], Jaana Ekojärvi[1], Igor Pavlov[3], Tatjana Morozova[4] and Kari Korhonen[1]
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We determined the decomposition rate (DR) of spruce wood by isolates of *Heterobasidion parviporum* at various temperatures. Sixty three *H. parviporum* isolates originating from geographically distant and climatically varying environments (Finland, Denmark, Italy and Central Siberia) were cultivated at eleven temperatures ranging from -4°C to 33°C. Saw dust of Norway spruce (fresh sapwood and heart wood) was used as the only substrate and DR was determined as the production of carbon dioxide. Optimal temperature for decomposition varied considerably between the isolates and ranged from 20°C to 30°C. Average DR by all isolates was highest at 30°C and lowest at -4°C: 18 and 0.5 µmol CO_2/d/1g of wood (d.w.), respectively. Based on these results, we calculated that a temperature increase of 2°C, for instance, would rise the DR by *H. parviporum* at winter temperatures (-4° – 0°C) by 50%, at spring and autumn temperatures (0 – +6°C) by 30% and at summer temperatures (6° – 20°C) by 20%.

The highest between-isolate variation in DR was observed at the extremes of the applied temperature scale, at 33°C and 0°C. At low temperatures (0 – +6°C) the DR by the isolates correlated negatively with the highest monthly temperature of the location from were each isolate originated (e.g. at 6°C $p = 0.000$, $n = 63$), i.e. isolates from warmer regions were less active at low temperature. Hence, these data suggest that *H. parviporum* has differentiated according to local climate.

Limitation in stump diameter for the spread of *Heterobasidion parviporum*

Anna Gunulf, LiYing Wang, Jonas Rönnberg and Torkel Welander
Swedish University of Agriculture Sciences

Earlier work has shown that even very small stumps can be infected by *Heterobasidion annosum.* Yet it is still not clear at what diameter the stump is starting to spread the disease. The objective of this study is to find out the lower limit of the stump diameter that can transfer *Heterobasidion parviporum* to the remaining surrounding trees.

Sixteen Norway spruce sites were chosen and divided into four groups according to site index, ranging from G20 to G36. On each site, 10 healthy looking trees within the same diameter class were felled, and the site diameter classes were 2-5cm, 5-8cm, 8-11cm and 11-14cm. The stumps were sprayed with an individual of *H. parviporum* (Rb175 (Jan Stenlid)). Five years later, the four biggest trees surrounding the "mother" stump were felled. Stump discs were taken both from the four trees and the "mother" stumps. Disc samples were taken to the lab and incubated. Colonies of *Heterobasidion* were isolated and cultured. Somatic mating tests were performed against Rb175 and homokaryotic testers of *H. annosum* and *H. parviporum*.

The result shows that infection of Rb175 spreads regardless of stump diameter. There is an indication that the probability of getting infected increases as the diameter increasing, except for diameter class 2-5cm exceeding that of diameter 5-8cm. There is also an indication that site index has a negative relation to the number of infected trees, i.e. the higher the site index is, the lower number of infected trees, except for a low number of infected trees in G20-G24 (the lowest site index group in the study). The percentage of adjacent trees being infected or not by Rb175 is almost similar in terms of diameter, growth rings and distance from mother stump. Noteworthily, there is no big difference in the average diameter of surrounding trees among different diameter classes of the mother stumps.

Heterobasidion parviporum can infect and spread to adjacent trees from a stump as small as 2 cm in diameter. So cautions should be taken during a precommerial thinning. Consequently, carring out stump treatment or thinning in a season when spore load is low is advised.

Occurence of fruit bodies of *Heterobasidion* on *Picea abies* in Latvian forests

T.Gaitnieks[1)], B.Stivriņa[1)], K.Kenigsvalde[1)], K.Korhonen[2)] & N. Arhipova[1)]

[1)]Latvian State Forest Research Institute "Silava", [2)]Finnish Forest Research Institute

Heterobasidion root rot causes serious economical losses in Latvian forests. On average, ca. 23% of *Picea abies* trees are decayed, mostly by *Heterobasidion* spp. Infection of fresh stumps and root wounds by *Heterobasidion* spores is greatly dependent on the amount of fruit bodies occurring in neighbouring forests. In this work our aim was to estimate the amount of fruit bodies on different types of spruce wood colonized by *Heterobasidion* and left in the forest.

Fruit bodies were measured on 367 spruce logs, 100 windthrown trees and 123 stumps in different forest types. The stand age at the time of cutting or windthrow varied between 34 and 139 years, and the inventory of fruit bodies was carried out 3 - 6 years later.

It was found that mean sporulating area of *Heterobasidion* fruit bodies per 1 m^3 of decaying logs was 58 dm^2 in forest types with abundant ground vegetation cover and 23 dm^2 in forest types with sparse vegetation. Fruit bodies developed above the soil on standing stumps only in forest types with abundant ground vegetation cover. On windthrown trees with uplifted root system, 68% of fruit bodies were on stem and 32% on roots (average from all forest types). On average, total surface area of fruit bodies on a windthrown spruce was 6.7 dm^2 on the stem and 4.3 dm^2 on roots. Fruit bodies were found also on spruces that were windthrown more than 10 years ago.

On the basis of our data it is recommended to remove spruce logs infected by *H. annosum* from forest. It would be important to remove also infected stumps with partly uplifted root system, at least on sites where the number of such stumps is high.

Potential of *Chondrostereum purpureum* as a sprout control of birches in Finland

Jarkko Hantula[1)], Henna Vartiamäki[1)], Pekka Maijala[2)], Rimvys Vasaitis[3)], Leena Hamberg[1)], Irja Löfström[1)] & Antti Uotila[2)]
[1)]Finnish Forest Research Institute, [2)]University of Helsinki, [3)]Swedish University of Agricultural Sciences

Hardwood sprouting is a major problem in forest regeneration, electric power line management and road sides; as well as in urban forests. In this work we aim to develop a biological control agent based on *Chondrostereum purpureum*, a common pathogen of hardwood stumps.

In a genetic diversity study we observed that *C. purpureum* in Finland and Lithuania was highly divergent, but did not show local differentiation or clonal structure. Despite this, a threefold variation was observed between eight isolates tested for their control efficiency against birch sprouting; the best isolates killed ca 80% of stumps. When correlated to the analysis of wood-decaying enzymes, it turned out that laccase activity (and perhaps also Mn-peroxidase) would be linked to the control efficacy. In Finnish conditions the treatment in the early summer seemed to be more efficient than late summer. Also the sensitivity of pruned birches was highest in May. Finally, analyses of *C. purpureum* treatment on aspen and rowan decreased stump sprouting.

As a conclusion, the biological control in Finland seems to be a promising approach towards hardwood sprouting. However, more efficient isolates should still be obtained before commercialization.

Fungi for biocontrol of *Robinia pseudoacacia,* an invasive tree species in Lithuanian coastal ecosystems

Vaidotas Lygis[1]*, Remigijus Bakys[2], Daiva Burokiene[1], Rimvydas Vasaitis[2]
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An invasive black locust (*Robinia pseudoacacia*) has been acknowledged as largely aggressive and unwanted tree species in the Curonian Spit of western Lithuania (Kursiu Nerija National Park), and immediate control measures of this plant need to be taken. *R. pseudoacacia* is difficult to control due to its rapid growth and clonal spread. In general, its management has concentrated on chemical control, however usage of herbicides is prohibited in the National Park, so the main aim of our study was to investigate possibilities to use a biological control method - mycoherbicides. The experiment has been started in July 2009 in young (up to 10-yr-old) regeneration of *R. pseudoacacia* growing on a burned site in Smiltyne Forest District (Kursiu Nerija National Park), where 23 permanent study plots have been established. In every plot, 50 trees have been cut and stumps were treated with a decay fungus *Chondrostereum purpureum* which is known as an effective mycoherbicide against many deciduous trees. Two formulation types (water suspensions of fungal mycelia mixed with two different drying out-preventing media: i) xanthan gum, and ii) AgroAquaGel®) were applied aiming to find the one which helps the fungal establishment best. First signs of the mycoherbicide effect (killed sprouts and root suckers) are expected in 1-2 years.
In addition, wilting *R. pseudoacacia* twigs showing symptoms of fungal infections have been collected around our study sites and isolation of the associated fungi was performed. Possibly pathogenic species will be tested in new inoculation experiments and the most promising ones will be used for a broader stump treatment experiment together with known pathogens of the black locust: *Diaporthe* (*Phomopsis*) *oncostoma*, *Phellinus robiniae* and *Trametes robiniophila.*

Rot-causing Basidiomycetes in Gray Alder *Alnus incana* (L.) Moench. in Latvia

Natalija Arhipova[1)], Talis Gaitnieks[1)], Jan Stenlid[2)], Rimvys Vasaitis[2)].
[1)] LSFRI"Silava"; [2)] Swedish University of Agricultural Science.

There are very few data about fungi, which can cause stem rot in *Alnus incana*. In most countries grey alder is not of high importance in silviculture, and is treated as a "weed" species. During the last years *Alnus incana* timber is being increasingly used for making packing materials in Latvia, and thus studies of rot causing fungi in wood of grey alder had raised an interest.

We collected wood samples (borer cores) using Presler borer from 431 living *Alnus incana* trees and 227 freshly cut stumps. All samples were sterilised in flame and put on Hagem agar media. 658 samples of *Alnus incana* wood yielded 1344 isolations representing 151 fungal species. All Basidiomycetes (40 species in total) were grouped into morphological groups; microscopical and molecular identification of each group were performed.

Different stages of decay (discoloration, hard or soft rot) were found in 71 % of all sampled *Alnus incana* trees. The most frequently isolated basidiomycetes in decayed *Alnus incana* trees were *Chondrostereum purpureum* (9.8%), followed by *Merulius tremellosus* (3.3%), *Armillaria cepistipes* (2.3%), *Bjerkandera adusta* (2.3%) and *Inonotus radiatus* (2.0%). The most common basidiomycetes in *Alnus incana* stumps were *Chondrostereum purpureum* (7.9%), *Inonotus radiatus* (6.2%), *Cylindrobasidium evolvens* (3.1%), *Bjerkandera adusta* (2.6%) and *Merulius tremellosus* (2.2%). *Bjerkandera adusta* also were found in 1.6% of healthy looking wood samples.

Chondrostereum purpureum as a biocontrol agent against sprouting of deciduous trees

R.Bakys, R.Vasaitis, J.Stenlid. Dept. Forest Mycology & Pathology, Swedish Univ. Agric. Sci., SE-750 07 Uppsala, Sweden.

The aims were to investigate: i) the efficiency of three *C. purpureum* strains as a biocontrol agent against sprouting of birch; and, ii) impact of felling season to sprouting energy of the stumps.

Two biological control experimental sites were established in Sweden and three sites in Lithuania during 2003 and 2004. Two experimental sites where placed under electric power lines (both in Sweden), one site in pure silver birch sapling stand and two remaining sites in mixed silver birch and Norway spruce stands. Water - mycelial suspension of three *C. purpureum* and one *Bjerkandera adusta* strains were applied to freshly cut birch stumps at four different application dates during the growing season - beginning of June, July, November and middle of August. All birch trees were 10 – 30 years old. A total of 3600 stumps were treated (including pure water controls), which were organized in repeated blocks of 20 – 25 stumps. Sprouting in Lithuanian experimental sites was assessed three times – 1, 3 and 5 years after the treatment. Experimental sites in Sweden were evaluated twice – 1 and 3 years after the treatment. The results showed pronounced differences of applied *C. purpureum* strains in ability to prevent sprouting (32.7 % - 76.5 % of stumps). Felling season had a pronounced impact on sprouting - the November cuttings produced the greatest number of sprouts while the June cuttings produced the fewest. In Swedish experimental sites larger number of re-sprouted stumps was found than in Lithuanian sites. After three years following treatment, sprout mortality was significantly higher than that observed after one year, and it remained so (even increased slightly) after five years, indicating persistent effect of the treatment.

External mycelium of ectomycorrhizal fungi within Heterobasidion genets

Taina Pennanen, Elina Lemström, Minna Sinkkonen & Tuula Piri
Finnish Forest Research Institute

The annual economic losses due to root and butt rot caused by *Heterobasidion parviporum* and *H. annosum* s.str. in Europe are estimated to at least 790 million euros. Intensive human activities in the forests are considered to be the main reason for the increased incidence of this most severe forest disease in Northern Europe. We posed a hypothesis that the impaired competitive status of external mycelia of ectomycorrhizal (ECM) fungi due to forest cuttings is one of the mechanisms behind the dominance of saprotrophic fungi *Heterobasidion* in managed boreal forests. Our aim is to compare the community composition of ECM external mycelia within aggressively expanding *H. parviporum* genets in spruce stands with that of *H. parviporum*-free patches.

In vitro, we have observed direct antagonism against *H. parviporum* and *H. annosum* s.str. by five of the tested 48 ECM pure cultures. The same strains belonging to the species *Paxillus involutus* and *Phialophora finlandia* were also able to protect young Norway spruce seedlings from lethal *Heterobasidion* infection in the laboratory. Currently we are analyzing community of ECM external mycelium in the seriously diseased Norway spruce stands.

Red band needle blight in Finland
Current situation

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Red band needle blight is caused by *Mycosphaerella pini* Rostrup (1957), also known as *Scirrhia pini* A. Funk & A.K. Parker and its conidial state is *Dothistroma septosporum* (Dorog.) M Morelet. First symptoms of red band needle blight are yellow spots on needles which turn later on brown. Red-brown coloration, commonly associated to the disease is caused by dothistromin which is a potent and broad–spectrum toxin and is responsible for the characteristic necrotic lesions and red bands on needles. Reproduction happens mostly asexually by conidia during the growing season in moist conditions, in Finland from May to October. Needles of all ages are infected by *D. septosporum*. If the infection is strong, needles can fall down during the same growing season, but more often they stay on branches to the next season.

In Finland symptoms of red band needle blight were recorded first time in autumn 2007 and in spring 2008, when acervuli and germinated conidia were found. There was more needlecast in 2008 than 2009. Red band needle blight has not yet caused serious defoliation in *P. sylvestris* and seems to restrict mostly to dense stands. Cold tolerance test has been made in test chambers to the needles with fresh acervuli. No clear differences could be observed in the germination of conidia after the different treatments. Climate change may have a positive impact to it's occurrence. When the weather is more humid and rainy and the growing seasons longer than until now, that can favor the experience of red band needle blight.

Fungal flora of Scots Pine needles, variation with altitude and latitude

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We are interested in how the fungal flora of Scots pine needles in Sweden varies with altitude and latitude. The number of retained needle sets varies with altitude and latitude and we also want to see if there is variation in the composition of the fungal communities of needles of different age, and if this variation can be connected to altitude and latitude. In August and September needles were collected from four different altitudinal gradients, the highest up to 600 meters. These gradients also make up a latitudinal gradient through Sweden. Needles were collected from the middle of the crown, from all retained needle sets.

Fusarium avenaceum and *Picea abies* container seedlings/ light and winter storage conditions, preliminary results

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Abstract

Fusarium sp. involved pathogenic species causing damage also in forest nurseries. It is frequently reported about *Fusarium* causing damping-off but there are some reports concerning winter storage damage. The aim of this study was to determine if *Fusarium*-like fungus, isolated together with *Herpotrichia nigra* from damaged spruce seedlings, could cause damage. This *Fusarium*-like isolate had an ITS-sequence identical to that of *Fusarium avenaceum* in GenBank; and was therefore tentative identified as *F. avenaceum*.

Spore production of *F. avenaceum* was tried on agar media, in liquid culture and on barley corn x needle homogenate media. The latest was used in the experiment. The temperature dependence of spore germination was examined on agar in Petri dishes. The spores germinate at 0^{o}C but most quickly at 25^{o}C.

First year spruce seedlings were grown in July-October in shade or in open field (light). The inoculation of seedlings was made monthly in July-October. Seedlings were winterstored in freezer or outdoor storage and checked after winter. Disease occurred only slightly in the following spring. Symptoms were dead needles or dead spots on needles. Isolations were made from them resulting *Fusarium* and/or other fungi.

The amount of damage at the top quarter of shoots was effected by winter storage factor, more damage occurred in seedlings that were winterstored in freezer than in those seedlings that were storaged outdoors. The effect of the light factor was clearest at the other shoot quarters, damage occurred more in seedlings grown in light than in shade grown seedlings. From the effects of the interactions the treatment (inoculation/control) x winter storage was clearest, inoculation effected more clearly in freezer than outdoor storaged seedlings.

Altogether the damage was slight and in these conditions *Fusarium avenaceum* was weak pathogen.

Establishment of ectomycorrhiza inoculated seedlings of *Pinus sylvestris* on coastal dunes following forest fire

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Reforestation of coastal dunes following forest fires may often result in low survival rates or even complete loss of outplanted seedlings. On such sites, low water and nutrient availability, damages caused by pathogens and insects, and lack of natural ectomycorrhizal (ECM) inoculum in the soil may negatively affect establishment and survival of newly outplanted tree seedlings. The aim of this study was to test the effect of artificial ECM inoculation of seedling roots on establishment and survival of tree seedlings on sandy coastal dunes after the forest fire.

Experimental plantation of *Pinus sylvestris* was established in spring 2007, one year after a large forest fire on coastal dunes of the Curonian Spit in western Lithuania using standard, bare-root nursery-cultivated seedlings. Inoculation of seedling roots with ECM fungi was done in two ways: 1) during the preceding growing season in the forest nursery by watering seedling roots with basidiospores of *Suillus luteus*; 2) immediately before the outplanting with vegetative mycelium of *S. luteus* and *Cenococcum geophilum*, pre-grown on sterile filter papers. In this case, each root system was covered with a filter paper containing viable mycelium, supplemented with a layer of moistened peat-sand mixture and wrapped in a paper towel. Three different ECM treatments (two with *S. luteus* and one with *C. geophilum*) and the non-inoculated control treatment were established in rows in 16 replicates on 2ha area. A total of 10,000 seedlings have been outplanted at the density of 5,000 seedlings/ha or 2,500 seedlings per each treatment.

After the first growing season, seedling survival was poor in all the treatments, ranging from 5% in control to 30% in the treatment of *C. geophilum*. In general, pine seedlings inoculated with ECM fungi showed significantly (χ^2 test, $p<0.0001$) better survival than seedlings in non-inoculated control. Seedlings inoculated with the vegetative mycelia of *C. geophilum* and *S. luteus* showed significantly (χ^2 test, $p<0.0001$) better survival (30 and 27%, respectively) as compared to seedlings inoculated with the basidiospores of *S. luteus* (10%). Persistence of the inoculated ECM fungi on seedling roots and infections caused by soil pathogens is under investigation.

In conclusion, artificial inoculation of pine seedlings with ECM fungi prior to their outplanting using selective fungal species/inoculation method can be used to promote reforestation of nutrient-deficient post-fire sites such as sandy coastal dunes.

Resin-top Rust Disease of Scots Pine caused by *Cronatium flaccidum*/*Peridermium pini* in northern Sweden

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In Norrbotten the most northern part of Sweden an outbreak of resin-top disease was reported in 2003. The disease was widespread mainly in young pine plantations.
In 2007 SLU carried out a forest damage inventory of the disease in the coastal area of the county of Norrbotten and in 2008 the inventory was widened to the whole area of the counties Norrbotten, Lappland and Västerbotten (Wulff and Hansson 2009). In total 130 000 ha young pine stands are affected by resin-top disease and within that area 33 000 ha ≥ 10 % of the stems are attacked.
An inventory in a progeny trial 2006 revealed wide genetic differences: The progeny from the most resistant plus trees exhibited no damage at all, whereas 75-100 % of the progeny from the most susceptible plus trees were found to be infected (Persson, Barklund and Andersson 2008).
The progeny trial was planted 1985 and location of stem injuries were found mainly on the 1998 internodes but also to some extent in the internodes of 1996 about 30 % of all trees in the stand investigated were diseased. With PCR-methods we could determine that the form *Cronartium flaccidum* occurred in the trial. That was surprising as the old truth was that *Peridermium pini* was supposed to be found in the north. However, to prove the existence or non-existence of *P. pini* was much more challenging. To study *C. flaccidum* and *P.pini* we adapted 7 microsatellites from other sources. It seems as it is possible to distinguish between mainly *C. flaccidum* and mainly *P. pini* populations by looking at the relative abundance of homozygotes. Both forms of the rust should than appear in the area of the disease.

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The effect of poplar leaf rust on frost damage of hybrid poplars

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Black cottonwood (*Populus trichocarpa*) was introduced from Alaska in Iceland in 1944. It became a popular tree species in gardens and parks. After frost damages in unusually hard spring frost in 1963, more clonal material from oceanic climate areas in Alaska was imported. After 1990 the forestry sector became interested in using poplars in mixture with conifers in afforestation. The yearly planting of poplars in forestry is now 6-8% of the total planting material. The increased interest in biofuels and carbon sequestration could further increase the use of poplars. In 1999 the poplar leaf rust (*Melampsora larici-populina*) was discovered in Iceland. As it seemed to be severe on black cottonwood, it was decided to start a breeding program in order to produce new clones with greater resistance against the rust. The crossing experiments were performed in 2002-2006. The progeny have been planted in trials in all parts of the country. The aim is not only rust tolerance, but also to get new clones which are growing well and are tolerant against spring and autumn frost damage. It seems that very few of the progeny clones have a good resistance against rust. In 2007 it was decided to try to produce poplar hybrids with fast growth and a good rust resistance. Flowering branches of two female clones of *Populus deltoides* were brought from Belgium (originally from USA) and the flowers pollinated with male flowers from two Icelandic (alaskan) clones. The 376 progeny were planted out in a trial plot in Mógilsá. In 2008 the rust infection was estimated and in spring 2009 the frost damage on the sprouts was measured, as well as other parameters. The progeny of each mother tree were kept separated. The progeny of the first mother (S-333 from Michigan) were 120 and the second mother (S-627-6 from Vermont) yielded 256 viable progeny. Of the progeny of S-333 47% had badly frost damaged shouts in spring 2009 and 81% of them had rust in 2008. The rest was with little or no damage and 28% of them had rust in 2008. Of the progeny of S-627-6 68% had severe frost damage in 2009 and 48% of them had rust in 2008. The rest was with little or no damage and none of them had rust in 2008.

The results show that the progeny of the Vermont mother are only undamaged by frost if they are completely resistant against rust. On the contrary 28% of the undamaged progeny of the Michigan mother had rust in 2008. Experience shows that it can be risky to select clones with complete rust resistance (vertical resistance) because such resistance can suddenly break down. It should be considered to choose some of the clones with moderate rust resistance for further trials and selection work.

Some causes for increased leaf rust abundance in wastewater treated willow plantations

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Previous studies of willow leaf rust (*Melampsora epitea*) abundance have shown increased levels of rust on the leaves of plants irrigated with wastewater compared to non-irrigated willow plants. Since the reasons for this difference were not clear, additional studies were carried out to determine weather the variation is due to differences in plant density or in plant leaf morphology.

Results showed that in areas with higher shoot density the rust abundance was higher compared to sparser areas. However, this tendency was clone specific and significant only in case of willow clones which infection starts from the lower part of the canopy (e.g. *Salix viminalis* clones). Therefore, higher rust levels in irrigated parts, which have also more shoots, can be only partly explained by higher canopy density.

Specific leaf area (SLA), as a widely used leaf morphological trait, was significantly correlated to rust abundance. SLA was higher in case of leaves with more rust damages (irrigated) and lower in leaves that were less infected (non-irrigated). This shows that wastewater irrigation could also change leaf morphology and therefore cause higher susceptibility to willow leaf rust.

By correlating these results with our previous studies we can conclude that wastewater irrigation could significantly increase the leaf rust abundance on willow because of the change in leaf morphology. However, since rust abundance can also be significantly influenced by canopy density, these plantations should be established either with sparser plantation design or with willow clones that have smaller shoot numbers.

Morphological differences in uredinial stage of aspen rust, *Melampsora populnea*

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Abstract: Aspen rust, *Melampsora populnea* is known as a collective species having at least four different strains or forma speciales according the alternate hosts on which they form their asexual aecial stage. The species definition of rust fungi can be based only on the telial morphology. That is apparently similar in each of these four strains. Perhaps, for that reason uredinial stage has remained without detailed investigations. In the herbarium of our institute (HFR) I found *M. populnea* specimens with different types of uredinia.

A rust with very small uredinia on the lower leaf surface was most common. This type produce small, elongated spores with thicker wall at longer sides. It seems, that the small uredinia often dispersed around the lower leaf surface resemble the original description of *M. populnea.*. The second type has wider uredinia, often aggregated into tight groups. The third type form uredinia on the bark of current year, as well as on the bark of several year old shoots. Spores of the large aggregated uredinia and of those developing on bark are similar, almost round, larger than the spores in the first type, with considerable variation in size, the wall is thick and quite even around the spores. The detailed descriptions of aggregated uredinia and those appearing on aspen bark are lacking.

Entomological problems for forest regeneration in clear-cuts and burned areas of Forest Steppe and Steppe of Ukraine

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Results of investigations in the clear-cuts and fire-sites in the pine stands of Forest Steppe and Steppe of Ukraine give possibility to list insect species, which are dangerous for forest edges, forest plantations and timber at colonizing, propagation and maturating feeding. Advisability of some forest management operations are discussed.
Threat of foliage browsing insects mass propagation, crown damage by xylophagous insects during maturation feeding, pathogens vectoring, additional weakening and colonization of trees increase at the borders with clear-cuts and burnt areas.
Larvae of Scarabaeidae are one of the main causes of seedlings mortality, *Melolontha hippocastani* (Fabricius, 1801) and *M. melolontha* (Linnaeus, 1758) dominate in the Forest-Steppe zone, and *Polyphylla fullo* (Linnaeus, 1758) and (*P. alba* Medvedev, 1951) dominate in the Steppe zone.
Artificial pine plantations and natural regeneration are damaged by maturation feeding of *Hylobius abietis* (Linnaeus, 1758)*, Hylastes angustatus* (Herbst, 1793), *Hylastes ater* (Paykull, 1800), *H. opacus* (Erichson, 1836) and *Hylurgus ligniperda* (Fabricius, 1787), are colonized by the last four species as well as are infected by pathogens being vectored by these insects.
Coarse woody debris can be colonized by stem pests which are dangerous for healthy forest in the case, if these insects complete development before decrease the relative humidity under the bark. Burning of coarse woody debris has negative influence on soil quality, biodiversity and carbon cycle.
Dead trees with relative humidity of wood below 30 % can't be the substrate for stem insects which are dangerous for conditionally healthy and weakened trees. Therefore such trees must not be felled at the aim of forest protection.

Causes of damage to *Pinus sylvestris* L. plantations on old burnt forest area in Ukrainian Steppe

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Difficulties of creation of pine plantations in the burned areas are connected with poor soils (sands), climatic factors (drought), complex of insect pests and diseases, as well as breach of planting technology.
Plantations in some plots damaged by forest fire of 1995 were replanted or complemented for several times because of severe mortality of transplants.
We studied the causes of mortality of pine plantations of different age – 1 – 3, 4 – 6 and 7 – 10 years old, considering transplants of four groups – visually viable, lightly weakened, severely weakened and dead. Living transplants were examined without destruction, perished plants were turned up, and both aboveground and underground parts were examined.
The part of transplants in good condition increased with age and is 24.7; 31.9 and 45.3% for 1 – 3, 4 – 6 and 7 – 10 years old plantations.
Damage by *Melolontha sp.* larvae was the cause of mortality for 35.8 % of transplants in plantations of 1 – 3 years old, 17.9% and 4.8% of transplants of 4 – 6 and 7 – 10 years old respectively.
Considerable part (24.8 – 42.8 %) of transplants in 3 – 10 years old plantations was populated by stem insects, mainly Coleoptera: Scolytidae and Curculionidae. *Hylurgus ligniperda* (Fabricius, 1787), *Hylastes angustatus* (Herbst, 1793), *Hylaster opacus* (Erichson, 1836) (Coleoptera: Scolytidae), *Pissodes notatus* F (Coleoptera: Curcilionidae) were the most abundant.
Signs of diseases were observed in 8.7% of dead, 7.5% of severely weakened and 7.8% of lightly weakened transplants of 1 – 3 years old. In the age of 4 – 6 years old 0.5% of dead, 0.5% of severely weakened and 8.7% of lightly weakened transplants had signs of disease. In the age of 7 – 10 years old 5.8% of severely weakened and 12.4% of weakened transplants had signs of disease. Conidial stage of *Leptostroma pinastri* Desm (anamorpha) and later apothecium of *Lophodermium pinastri* Chev (teleomorpha) were detected on pine foliage.
Some transplants had no central root and were survived only due to lateral roots. 96% of such plants were colonized by *Pissodes notatus* F и *Anthaxia quadripunctata* L. (Buprestidae).

Population structure of *Cerrena unicolor*, wood-decay polypore symbiotic with the horntail *Tremex fuscicornis*

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It is known that horntails might have a pronounced impact on population structures of their symbiotic wood-decay fungi. In northern Europe, for example, studies on *Amylostereum areolatum* had revealed prevailing spread of the fungus by a horntail *Sirex juvencus*. Here, populations of *A. areolatum* consist of large widespread dispersive clones with low genetic variation. By contrast, populations of related fungus, *A. chailletii*, vectored by horntail *Urocerus gigas*, are composed mainly from geneticaly diverse individuals, and only a few small locally spread dispersive clones are found, indicating to limited transfer of the fungus by the insect. The aim of this work was to check an impact of horntail *Tremex fuscicornis* on population structure of its symbiotic wood decay fungus *Cerrena unicolor*. A total of 30 heterokaryotic isolates of *C. unicolor* were derived from wood beyond sporocarps, and their geopgraphic locations mapped. The studied population sample was collected from Czech Republic, mid- and north- Sweden, and Finland, thus represented a wide range of spatial scales. All isolates were subjected to vegetative compatibility tests by pairwise confronting them in all possible combinations on 9 cm Petri dishes containing agar medium. Mycelial reactions were compared with those of self-pairing controls.

Colonization of trees and coarse woody debris by xylophagous insects in the clear-cuts after fire

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In August 2008, in Izum Forest Enterprise (Kharkov region) about 2000 ha of pine stands were damaged by fire. In the part of area, burnt trees were felled and removed. In the other part of burnt area the trees were left standing or felled and kept as logs.
We tried to study
– is this a threat of colonization by xylophagous insects of different kinds of substrate (living trees, log decks and coarse woody debris)?
– can the insect that emerge from dead trees, coarse woody debris and log decks colonize living trees?
– what manner of storage of logs provides the best conditions for propagation of xylophagous insects?
One of the tasks was to determine what to do with the trees damaged by fire – to leave standing or to fell.
In the burnt forest, standing sample trees were numerated, their sanitary condition and level of damage by fire were registered.
In November 2008, branches (4–7 cm in diameter) and logs (8–25 cm in diameter) were put on the soil and on the decks in different parts of the burnt area.
In May, June and August of 2009 sanitary condition of sample trees was assessed, living and dead trees as well as log decks and coarse woody debris were examined for entrance and exit holes of insects, egg and larvae galleries were counted, population indices evaluated. Species composition for xylophagous insects was determined, their stages and instars, depth of wood damage. Temperature of air, soil and space under bark as well as relative humidity were measured to determine optimal conditions for survival and development of different xylophagous insects.

Fungi Vectored by the Bark Beetle *Ips typographus* Following Hibernation Under the Bark of Standing Trees and in the Forest Litter

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The bark beetle Ips typographus has different hibernation environments, under the bark of standing trees or in the forest litter, which is likely to affect the beetle-associated fungal flora. We isolated fungi from beetles, standing I. typographus-attacked trees, and forest litter below the attacked trees. Fungal identification was done using cultural and molecular methods. The results of the two methods in detecting fungal species were compared. Fungal communities associated with I. typographus differed considerably depending on the hibernation environment. In addition to seven taxa of known ophiostomoid I. typographus-associated fungi, we detected 18 ascomycetes and anamorphic fungi, five wood-decaying basidomycetes, 11 yeasts, and four zygomycetes. Of those, 14 fungal taxa were detected exclusively from beetles that hibernated under bark, and six taxa were detected exclusively from beetles hibernating in forest litter. The spruce pathogen, Ceratocystis polonica, was detected occasionally in bark, while another spruce pathogen, Grosmannia europhioides, was detected more often from beetles hibernating under the bark as compared to litter. The identification method had a significant impact on which taxa were detected. Rapidly growing fungal taxa, e.g. Penicillium, Trichoderma, and Ophiostoma, dominated pure culture isolations; while yeasts dominated the communities detected using molecular methods. The study also demonstrated low frequencies of tree pathogenic fungi carried by I. typographus during its outbreaks and that the beetle does not require them to successfully attack and kill trees.

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Growth strategy of *Heterobasidion parviporum* within stems of Norway spruce

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In Norway spruce, a fungistatic reaction zone with high pH and enrichment of phenolics is formed in the sapwood facing heartwood colonized by the white-rot fungus *Heterobasidion parviporum*. Fungal penetration of the reaction zone eventually results in expansion of this xylem defense. To gain knowledge about mechanisms operative at heartwood and reaction zone colonization by the pathogen, hyphal growth and wood degradation were investigated with real-time PCR, microscopy and comparative wood density analysis in naturally colonized trees with extensive stem decay. The hyphae associated with delignified wood at stump level were devoid of any extracellular matrix, whereas incipient decay at the top of decay columns was characterized by a carbohydrate-rich hyphal sheath attaching hyphae to tracheid walls. Pathogen DNA amount peaked within aniline wood, a narrow darkened tissue at the colony border apparently representing a compromised region of reaction zone. Vigorous production of pathogen conidiophores occurred in this region. Colonization of aniline wood was characterized by hyphal growth within polyphenolic lumen deposits of tracheids and rays, these hyphae being encased fully in a carbohydrate-rich extracellular matrix. Taken together, these data indicate that interaction of the fungus with reaction zone involves a local concentration in fungal biomass that forms an efficient translocation channel for nutrients. Finally, the enhanced production of hyphal sheath may be instrumental in lateral expansion of the decay column beyond the reaction zone boundary.

A rapid method for testing growth rate of *Phlebiopsis gigantea* isolates

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Biological control agent Rotstop®, containing oidia of *Phlebiopsis gigantea*, is used in northern Europe to control *Heterobasidion* root rot. Rotstop was registered in Latvia in 2006. Large-scale use of a single Rotstop genotype in Latvian forests raises the question about its effect on the genetic diversity of local populations of *P. gigantea*. Since 2007 we have tried to find effective Latvian isolates of *P. gigantea* which could further be used for the stump treatment. Testing the efficacy of many isolates of *P. gigantea* against *Heterobasidion* in stumps is laborious. However, the efficacy of an isolate seems to be in positive correlation with its growth rate in wood. Hence many isolates of *P. gigantea* can relatively easily be screened preliminarily by measuring their growth rates in stem pieces (billets) of spruce and pine. The inoculation of billets is usually carried out by spraying oidial suspension of *P. gigantea* on fresh cutting surface. We used two inoculation methods for the growth rate testing of Latvian isolates: spraying and a pit method. In the latter method the oidial suspension is placed in a pit or groove made on the cutting surface of the billet. The results obtained with both methods were approximately similar. The greatest advantage of the pit method is that it is possible to test up to 15 isolates in the same billet (practical maximum with spraying is ca. 6) and to control more exactly the amount of the inoculum.

Differential screening of *Phlebiopsis gigantea* isolates for traits associated with biocontrol of the conifer pathogen *Heterobasidion annosum*

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Abstract

Stump treatment with the saprotrophic fungus *Phlebiopsis gigantea* has been widely used as the preferred method for the control of the root rot disease caused by *Heterobasidion annosum* sensu lato. Very little is however known about the traits that are important for the biocontrol action. In the present study, 64 isolates of *P. gigantea* from diverse geographical sources were screened for growth rate, laccase production, wood decay capability and antagonistic ability under *in vitro* conditions. The data was statistically analyzed using principal component analysis and multiple regression, where correlation between different variables was tested. Results revealed significant variations in the growth and antagonistic ability of the 64 isolates. The nutrient source had a significant effect on the outcome of interactions; 90% of the *P. gigantea* isolates were able to replace *H. annosum* on wood media whereas the corresponding value for glucose-rich Hagem media was 4%. The result also showed that growth rate on Norkrans media with wood as sole carbon source correlated with growth rate on Norkrans with ferulic acid, a lignin precursor ($P = 0.08$), growth rate on xylan, a hemicellulose ($P = 0.0001$) and percentage weight loss in pine ($P = 0.01$). Interaction in sawdust correlated with high wood degradation capability in pine and spruce with *P*-values ($P = 0.01$, $P = 0.03$) respectively, high growth rate in xylan ($P = 0.01$), laccase production ($P = 0.08$), interaction in Hagem ($P = 0.01$) and mean growth rate at 10 ˚C ($P = 0.001$). The results show that the antagonistic interaction is partly dependent on the ability of the two fungi to degrade the different structural components of wood so as to gain access to carbon which is the primary source of nutrient for their survival.

Key words: biocontrol, antagonism, wood degradation, *Heterobasidion annosum*, *Phlebiopsis gigantea*.

Recent advances on the control, epidemiology and detection of *Heterobasidion annosum* in *Picea abies* in Scandinavia

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The fungus *Heterobasidion annosum* (Fr.) Bref. causes root and butt rot in *P. abies*, resulting in economic losses for the forestry sector in the Scandinavian countries. Regular forestry operations such as thinning and final felling facilitate the spread of this fungus. Airborne spores land on freshly created stumps and spread towards healthy trees through root grafts. Winter thinnings may reduce the risk of infection as the spore load is generally low in that season. Summer infection may be diminished by spreading urea on the stump surface hence raising the pH towards toxic levels for *H. annosum*, or by spreading a spore suspension of the rot fungus *Phlebiopsis gigantea* (Fr.) Jülich in order to out-compete subsequent *H. annosum* airborne infections. Long term reliability of these control methods when performed as in normal forest conditions needs to be elucidated. Tree growth reduction has been observed associated with *H. annosum* butt rot. Growth reduction might result from an allocation of photosynthates in order to produce a reaction zone to prevent fungal advance with the consequent reduction of secondary growth, however, this hypothesis has not been thoroughly studied yet. Growth reductions under a whole-stand perspective are poorly documented in the literature and need to be studied. Two *Heterobasidion* species cause butt rot in *P. abies* in Scandinavia: *H. parviporum* and *H. annosum s.s.* The epidemiology of these fungi has not been studied under a long term experimental perspective. Host- or substrate-specialization may be used as control method by creating conditions unfavourable to the pathogen and thus reducing its inoculum. Forest managers may be interested in having early information on the rot incidence in a stand prior to the felling age. Rot incidence at an intermediate point of the rotation can be used for predicting the rot incidence at the end of the rotation, future revenues can be estimated, and thus rotation period may be modified. The instrument Rotfinder® is currently commercialized as a non-destructive method of detecting rot in standing trees, but an independent assessment of its reliability is needed. Here we present the results of three different experimental trials, aiming i) to study the long term reliability of stump control methods when applied as normal forest operations; ii) study the growth reduction associated with butt rot iii) to study experimentally the epidemiology of *H. parviporum* and *H. annosum* in *P. abies* stands, and iv) to assess the accuracy of the Rotfinder instrument detecting rot in standing trees. The first objective was studied by comparing the rot incidence and the *H. annosum* population structure

between five treatments 13 years after their application: i) winter thinning, ii) summer thinning, iii) summer thinning with urea treatment (35% w/v) of the stumps, iv) summer thinning and treatment of the stumps with *P. gigantea* and v) no thinning. All treatments were performed in eleven stands located in Sweden. The stands were grouped as forest or agricultural land, depending on the use prior to plantation. The second objective was attempted by comparing the increase of tree diameter among the trees that were sampled in the treated stands. The presences of the characteristic olive green reaction zone and resin flow from trees were noted. The third objective was accomplished by artificially infecting spruce stumps with *H. annosum* and *H. parviporum* conidia after the first thinning in two first rotation stands, located in southern Sweden. The same isolates were randomly applied to different stumps within and between stands. Two infection regimes were applied in two plots per location: i) all stumps infected and ii) 50% of the stumps infected and the other 50% sprayed with a urea solution. *Heterobasidion* was re-isolated 15 years post-treatment from the surrounding trees and the collected isolates were matched to the original isolates by somatic compatibility tests and microsatellite profiling. *H. parviporum* and *H. annosum* were compared in terms of success of the artificial infection, number of trees colonised after 15 years and maximum distance covered from the original stump. For the fourth objective, we tested Rotfinder on 500 spruce trees corresponding to three different stands in central Sweden. Trees were measured with Rotfinder at three different stem heights (0.3, 0.6 and 1.3 m). After trees were felled, every measured section was inspected for the presence of rot and reaction zone. The stump control methods proved to be a reliable tool for minimizing airborne *H. annosum* infections. However, in previously forested stands, *H. annosum* infection seemed to be greatly determined by the rot coming from previous rotations. Both urea and *P. gigantea* provided similar protection levels, though the latter seemed more sensible to incomplete cover of the stump surface. Rotten trees with reaction zone had grown less than healthy trees. We could not detect differences in radial growth between rotten trees without reaction zone and healthy trees. *H. parviporum* presented a two-fold higher success in artificial infections that *H. annosum s.s.*, however, once established *H. annosum s.s.* was able to colonise a higher number of trees. *H. parviporum* infections were more successful in stumps with larger diameter. The infection success of *H. annosum* did not correlate with the diameter of the stump. The biological reasons for a diameter-dependent success of infection in the case of *H. parviporum* need to be investigated, since performing first-thinnings earlier could be tested as a control method for minimising the risk of *H. annosum* infection in first thinning. Sensitivity, specificity and accuracy of Rotfinder will be discussed.

Impact of ash decline in Denmark

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The future of ash (*Fraxinus excelsior*) as a forest tree species and amenity plant in Denmark is threatened by ash dieback, caused by the fungus *Chalara fraxinea*. Symptoms have been registered since 2003, but the problem became widespread in 2005, and since 2007 the impact has been severe both in forests and in the landscape. By now trees are attacked by secondary pathogens, mainly *Armillaria* sp., and whole stands are being cut down at once, or stepwise as the trees decline. Young ash stands up to age 40 years have succumbed quickly, but even old trees are slowly dying. As yet, no impact has been registered on wood quality of the timber, if no epicormic sprouts have formed on the stems. The forestry sector and timber industry are worried about the long term supply of ash wood. Planting of ash has almost stopped completely, and most young stands will not survive to produce harvestable timber. At present, the only possible solution seems to be development of resistant ash clones for future use.

***Fraxinus excelsior* shows genetic differences in susceptibility towards *Chalara fraxinea*, novel ash dieback.**

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The health and viability of European common ash is presently threatened by a novel disease presumably caused by the fungus *Chalara fraxinea*. Symptoms have been registered over the past few years originating in the north eastern parts of Europe and spreading towards the west, including Scandinavia. In Denmark assessment of clonal field trails have indicated the presence of genetic resistance in some *F. excelsior* clones. 40 clones were assessed at two locations (2007-2009). Additionally 101 half sib families were tested at two sites (2006-2009) and initial results identify a low frequency of clones and families showing significantly less symptoms. A new project aims to understand the plant-pathogen interaction and the underlying basis of the observed resistance.

Ash dieback in Norway

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The first registration of ash dieback caused by *Chalara fraxinea* in Norway was in 2008. Then it was spread over large areas of eastern og southern part of Norway. The year before we had extensive winter damage which could have camouflaged the dieback. In 2009 thousands of *Hymenoscyphus* fruitbodies were found under nearly all ash trees in eastern Norway and we have done spore sampling. At the west coast, where we not have observed damages caused by *C. fraxinea* fruitbodies of *Hymenoscyphus* cf. *albidus* has been collected. The sequence from these samples differs somewhat from *Hymenoscyphus* samples and *C. fraxinea* from eastern Norway.

Chalara fraxinea, biology and genetics

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The tree pathogen *Chalara fraxinea* has caused ash dieback since the early 90's. The disease has since then expanded its geographical distribution to most parts of the distribution of ash in Europe. How has this disease spread and how does it interact with its host? Is the disease intensity varying through the year? How to recognize the disease in a fast and reliable way? To obtain answers to these questions a number of projects are carried out:

- Genetic studies are made by use of seven biallelic microsatellite primers. Isolates from Sweden, Denmark, Finland, Lithuania, Germany, Poland, Hungary and Austria are evaluated. No geographical clustering could be distinguished so far when using Fisher exact-test. Work is in progress to include more isolates, and to extend the analysis.
- Visible lesion extension is studied by field observations of about 250 ash trees distributed on four localities. The trees are 5 -20 years old. Lesions are followed and measured every month, and health status of the tree is estimated. Preliminary results show that lesion extension can go on all over the year. However after almost two seasons of measurements it seems as the lesion extension is favoured in spring and summer: the highest number of lesions was active between April and July, and the longest extensions measured both in distance and area is seen between May and August.
- Metabolites produced by the fungus were examined. The toxin viridiol was isolated from *C.fraxinea* culture media. Ash-seedlings formed necrosis on leaves when treated with drops of the toxin. (Andersson et.al. 2009)
- A pair of molecular primers were developed in the ITS region and adjacent intron. Use of the primers makes it possible to detect *C.fraxinea* in plant material.(Johansson et.al. 2009)

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Phytophthora cactorum and some recently found, non-native pathogens in Finland

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The comparative ease and speed of international trade and travel have enabled pests to disperse around the world. The ornamental plant trade in particular has unwittingly trafficked alien Oomycetes such as pathogens in the genus *Phytophthora*. So far three *Phytophthora* species have been identified on nursery seedlings in Finland: *P. cactorum, P. inflata* and *P. ramorum*. Recently we found a new species from *Sorbus acuparia* seedlings producing hyphae and oogonia typical for *Phytophthora*.

In the mid-1990s, an epidemic of foliar rust on *Alnus glutinosa* and *A. incana* broke out in Finland. The pathogen responsible was the basidiomycete *Melampsoridium hiratsukanum.* Two other pathogenic fungi that have recently been detected in Finland are the ascomycetes *Dothistroma septosporum*, responsible for red band needle blight and *Chalara fraxinea*, responsible for ash decline. Typical symptoms of red band needle blight were first found on *Pinus sylvestris* in multiple locations during the summer of 2007 and 2008. Isolates from needle acervuli provided pure cultures of *D. septosporum.* The first signs of ash decline were observed in 2007 on *Fraxinus excelsior* from trees growing in its native range of southern Finland and the Åland archipelago.

Models of climate change suggest that introduced species will find it easier to establish in new areas. With time, we expect more non-native pathogens to be introduced or detected in Finland.

Vertical structure of ectomycorrhizal species composition in relation to soil phosphorus sources.

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Nutrient availability is a major factor limiting tree growth in dry, sand-dominated soils. Extensive N deposition along the Baltic coast makes phosphorus a potentially limiting nutrient in these ecosystems. In soils, P occurs in primary and secondary minerals as well as in organic form. The source of P, changing with depth, is likely to be an important factor shaping the ectomycorrhizal community because of different P use strategies by different fungi. Completed and on-going experiments will be reviewed We are currently investigating whether there is spatial correlation between ectomycorrhizal community composition and P form along a spatial gradient from pure sand (near the coast) to soil with increasing amounts of accumulated organic material. This work is being conducted within the framework of the EU project LITCOAST. Samples from 1-100 cm depth have been collected in a coastal forest north of Palanga, Lithuania along 5 x 100 m transects from coastal dune to soil with developed understorey vegetation and higher organic matter. pH and soil moisture profiles have been determined and ergosterol and DNA extracted for community profiling using 454 pyrosequencing
The results will provide novel information about P utilisation strategies in different mycorrhizal taxa, as well as new information about vertical stratification of different taxa, building upon a highly cited earlier article of ours (Rosling *et al,* 2003).

The ecology of *Peniophora junipericola* (Basidiomycota, Russulales)

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Peniophora junipericola J. Erikss. is locally common wood-rotting fungus on dead junipers (*Juniperus communis*) around the Baltic Sea. The aim of the present study is to give an overview of the ecology of *Peniophora junipericola*. The ecology was studied by measuring the diameter of the substrate, the stage of decay and the pH values of the infested twigs. The amount of precipitation was used for characterizing the localities. The results of the present study show that the fungus can grow on thin as well as on thick; on hard, newly dead branches and twigs as well as highly decayed twigs. Most of habitats are situated close to the sea, sometimes it can grow also in inland.

Particular characteristics of soil microbial communities in forest stands infected with *Heterobasidion parviporum* and *Armillaria* spp.

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University of Latvia

The study was undertaken to characterize soil microbial communities of forest stands infected with root rot fungus *Heterobasidion parviporum* Niemelä & Korhonen and honey fungus *Armillaria* sp. The objectives of the study were to characterize and compare soil microbial communities using following methods: estimation of the amount of cultivable microorganisms colony forming units (CFU) using plate count method; determination of typical fungal genera followed by calculations of Shannon – Weaver diversity index; extraction of total soil DNA; PCR amplification of the fungal nuclear ribosomal fragments of the obtained DNA followed by ARDRA and calculations of Shannon – Weaver diversity index; and quantitative PCR with universal fungal primers and *Trichoderma* spp. specific primers.
Two 40 years old spruce (*Picea abies* (L.) Karst*)* stands in *Oxalidosa* and *Myrtilloso-polytrichosa* forests, infected with *H. parviporum* were analyzed on Sod-podzolic soils (Cutanic, Stagnic Albeluvisols) and Illuvial humus podzol (Placic, Rustic, Albic, Folic, Stagnic Podzols) according to Latvian soil classification and FAO WRB soil classification. In each stand one sampling plot was established.
The forest infected with *Armillaria* sp. was 80 years old mixed forest stand in *Myrtillosa turf. mel.* forest on drained fen peat soil (Histosols). Dominant tree species was pine *Pinus sylvestris* (47 % from the area). Other tree species were birch (*Betula pendula* Roth), aspen (*Populus tremula* L.) and gray alder (*Alnus glutinosa* Gärtn.). In this stand three sampling plots were established.
Obtained data were compared with soils of healthy forest stands analyzed in other our investigation.
Infection of the analyzed forest stands by parasitic fungi is reflected by significant decrease of the number of CFU of filamentous fungi and by slight decrease of cultivable microorganisms CFU and fungal diversity (with exception of Shannon-Weaver diversity index of cultivable filamentous fungi of sampling plots infected with *Armillaria* sp.). In contrast infected stands with *H. parviporum* have relatively high proportions of fungal DNA from the total soil DNA and *Trichoderma* spp. DNA from fungal DNA, and also higher relative abundance of cultivable *Trichoderma* spp. in comparison with data about healthy forest stands from our previous publication. Soils in sampling plots infected with *Armillaria* sp. have decreased proportion of fungal DNA from the total soil DNA but increased proportion of *Trichoderma* spp. DNA from fungal DNA.

Scots pine Antimicrobial peptides (*SP-AMP*) gene expression is induced by exogenous glucan and mediated through salicylic acid and ethylene signalling pathways

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A novel family of antimicrobial proteins has been discovered, so-called Sp-AMP in *Pinus sylvestris* (Scots pine) (Asiegbu *et al*., 2003)*. Using a combination of Northern analysis and Real-time quantitative RT-PCR, we studied the expression of Scots pine *AMP* during challenge with either a pathogenic (*Heterobasidion annosum*), mutualistic (*Lactarius rufus)* or saprotrophic (*Stereum sanguinolentum)* fungi. The results indicate that *Sp-AMP* expression is induced during pathogen and non pathogen interaction; an increased *Sp-AMP* expression was observed at a very early stage when challenged with either the pathogenic, mutualistic or saprotrophic fungi. However, *Sp-AMP* expression is eventually attenuated at prolonged incubation with mutualistic or saprotrophic fungi but remained high with the pathogenic fungi. In a follow-up study, we investigated if *Sp-AMP* expression is provoked by the presence of fungal cell wall chitin, chitosan or glucans. All three compounds provoked strong necrotic reaction on the roots but only glucan induced the *Sp-AMP* expression. The study was repeated by inoculating Scots pine roots with yeast mutants devoid of chitin or glucan, increased expression of *Sp-AMP* was observed with the two yeast mutants only at 5 d.p.i (day post inoculation) but not at one d.p.i. Additionally, Scots pine roots were challenged with fungal material devoid of cell wall (protoplast), the results showed that only protoplast from the pathogenic and mutualistic fungi induce significant *Sp-AMP* expression. Together, our data suggests that several factors other than the fungal cell wall components play a role in the regulation of *Sp-AMP* expression. We conducted a complimentary study to investigate the possible mediatory role of methyl jasmonic acid, salicylic acid, ethylene and hydrogen peroxide responsive pathways in *Sp-AMP* regulation; our results indicate that *Sp-AMP* expression induced via SA- independent and ethylene signalling pathway and acts independently of methyl jasmonate and hydrogen peroxide signalling pathways. We will further investigate the regulation of other **defense**-related **gene** (Peroxidase and defensin *PsDef1*) in Scots pine and the *Arabidopsis* defensin (the closest homologue to *Sp-AMP*). The results will be discussed with reference to the possible role of AMP in defence against phytopathogenic fungi of conifer trees.

*Asiegbu F.O., Choi W., Li G., Nahalkova J., Dean R.A. (2003) FEMS Microbiology Letters 228: 27-31

Diversity of wood-inhabiting fungi in stems and roots of mountain pine (*Pinus mugo* Turra.) with special emphasis on root rot-causing basidiomycetes

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Curonian Spit, a site included into UNESCO World Heritage List, is a unique example of a landscape of sand dunes and forest that is under constant threat from natural forces and recreational pressure. Plantations of mountain pine (*Pinus mugo*) occupy 27% (1,850 ha) of forest stands of the Curonian Spit. Changing climate, high anthropogenic pressure, forest fires and diseases have an impact on mycobiota of the coastal mountain pines and the shift in community structure of wood-inhabiting fungi following those disturbances may be dramatic. The main aims of the present study were: i) to investigate and to compare communities of fungi that inhabit wood of approx. 100-year-old mountain pine of various condition, and ii) to investigate the occurrence and viability of the root pathogens *Heterobasidion* and *Armillaria* in root disease centres on burned and non-burned sites in *P. mugo* forest.

Fungal isolations were made from: i) stems of sound-looking trees (PS, 50 trees); ii) stems of trees killed by a forest fire (PB, 50 trees); iii) stems of trees killed by root pathogens 0-1 years ago (PR, 50 trees); iv) stems of trees killed by root pathogens 1-3 years ago (PO, 50 trees); v) stump surfaces of burned trees cut after the forest fire (SB, 112 stumps); vi) stump surfaces of non-burned trees (SC, 50 stumps); vii) roots of trees killed by the root-rot and burned by a forest fire (RB, 230 root systems); and viii) roots of non-burned trees killed by the root-rot (RC, 40 root systems). Three types of wood samples have been taken: bore cores extracted from root collars (PS, PB, PR and PO), bore cores extracted from stump tops (SB and SC), and root pieces sampled at 10–30 cm depth in the soil (RB and RC).

A total of 1064 fungal isolates representing 77 taxa have been obtained from 632 wood samples. The two most frequently isolated fungi were cosmopolitan ascomycetes *Trichoderma viride* and *Penicillium* cf. *commune*. *Heterobasidion annosum* s.s., *Resinicium bicolor* and *Armillaria borealis* were the most commonly isolated basidiomycetes occurring only in dead wood (almost exclusively isolated from roots). Zygomycetes were isolated at relatively low rates. The highest frequency of isolation of the fungal taxa was found in SB and RC, while the lowest – in PR and PS. Species richness was the highest in PO and PB, while the lowest – in RB and PR. Fungal communities were moderately similar (in qualitative and quantitative terms) in pine roots on burned vs. non-burned sites, on stump surfaces of burned vs. non-burned pines, and in stems (root collars) of burned vs. sound-looking pines. However, the fungal communities found in stems (root collars) of pines attacked by the root rot (PR and PO) were clearly different from non-attacked ones (PS and PB). Consequently, the shift in fungal

community structure in pine stems (root collars) is more expressed following the attack of root rot fungi and subsequent decomposition than the burning.

H. annosum s.s. gave growth from 42.5% of root systems on non-burned, and from 15.2% of root systems on burned sites, thus forest fire in disease centres seemingly had reduced vitality of the pathogen. *A. borealis* was less commonly isolated (3–5% of root systems). Somatic incompatibility tests with 35 strains of *H. annosum* s.s., isolated from six disease centres, showed genetically diverse populations of the fungus even within small and compact areas (0.02–0.04 ha).

Keywords: biodiversity, disease centres, forest fire, fungal communities, mycobiota, persistence.

Multiproxial approach to the epidemiology of *Lophodermium* needle cast of Scots pine (*Pinus sylvestris* L.) in Estonia

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In difference from the practice in several European countries, the role of bare-root pine seedlings in forest regeneration of Estonia is still high, e.g. in 2004-2007 more than 70% of the Scots pine planting stock was grown in open-land nurseries. Consequently, the epidemic foliage disease *Lophodermium* needle cast of pine in this country is persistently a live issue. Additionally and generally, *Lophodermium* needle cast has a definite role in the natural selection of surviving and failing tree-individuals during the early stages of stand formation.

Epidemiological modelling of the disease should be useful for building up a better control strategy in nurseries and for better understanding of the role of the disease in the pine growth in forests. As a next step in the appropriate epidemiological investigations, we juxtaposed several proxies: (1) literature data about the epidemic occurrences of *Lophodermium* needle cast, (2) data of the diagnostic laboratory from 1972-1985, (3) data of the needle losses (from NTM - Needle Trace Method computations), (4) meteorological data, (5) GIV-s (yearly Gremmeniella Index Values, cf. Thomsen, 2009) for Estonia, (6) foliage monitoring data from the nearest international (ICP Forest) monitoring plots, (7) calamity years of herbivorous insect pests of pine, and annual growth rates of trees (from the NTM) at the post-epidemic (8) and post-calamity (9) years.

The poster presentation introduces the results of our investigations, concerning e.g. specification of the critical months and amounts of summer precipitation, needful for triggering of an epidemic in the natural conditions of Estonia, susceptible host age limits, comparison of the effects of *Lophodermium* needle cast and herbivorous insect pests on the height and radial growth of pine.

Control of Heterobasidion root rot by stump removal - preliminary results of the ongoing field trials in Finland

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The use of Norway spruce stumps to generate energy from final harvest areas has increased rapidly in recent years. In addition to utilizing an often-untapped source of forest fuel, stump removal has been proposed as a control measure of Heterobasidion root and butt rot. In order to maximize the benefits of stump harvesting and disease control, information from long-term experiments and practice is needed. In response to this need, several field experiments have been established in southern Finland: 1) Long-term field trials have been established (2003-2007) to evaluate the extent to which infected stump removal reduces the transfer of Heterobasidion root rot to the next rotation of spruce and pine trees. Disease development in the next rotation will be followed at least until the first thinning. 2) A study will determine the persistence and infectivity of *Heterobasidion* mycelium on broken root pieces remaining on the regeneration area following harvesting. 3) The risk posed by long-term storage of spruce stumps infected by *Heterobasidion* will be evaluated by examining the occurrence of fruiting bodies in spruce stumps kept in roadside storage for 1-5 years. The preliminary results have shown that *H. parviporum* is able to survive for at least for three years in buried root fragments. The fungus is also able to produce fruiting bodies while surviving on such roots. In stump piles, the first fruiting bodies appear on decayed stumps appear after two years of storage.

Spread of *Heterobasidion parviporum* in a managed Norway spruce stand containing uneven-aged trees

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Of the commercial tree species in Finland, Norway spruce regenerates best in the shade of taller trees and is therefore suited to a stand containing trees of different ages. In such stands with a history of selective harvesting, we detected *Heterobasidion* root rot in young (34% infected), intermediate (19%) and overstory (50%) trees. Decay among overstory trees will reduce the timber volume available for harvest in the near future, and infection of intermediate and young trees in the longer term. Furthermore, because of the importance of Norway spruce to boreal forestry, *Heterobasidion* root rot poses a considerable threat to the production of sound timber in continuous cover stands.
The spatial distribution of *Heterobasidion* genets within disease centres suggests that infection routes exist between different layers of adjacent trees and that this pathway may be common in uneven-aged spruce stands. Protecting residual trees and freshly-cut stumps from spore infection will be a critically important strategy for controlling *Heterobasidion* root rot. This is particularly true in forests where little or no disease is currently present.

Ectomycorrhizal community structure: The role of host tree genotype

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It is well established that resistance of forest trees to disease is under genetic control but our knowledge of the importance of host genotypic variation in mycorrhizal formation is currently limited. However, recent studies found marked variation in ectomycorrhizal fungi (ECM) communities among conspecific tree and the ability of tree individuals to form ectomycorrhizas with a single fungal strain has been demonstrated to have a genetic basis. These observations suggest that host genotype is an important factor in determining the associated ECM species composition, which may in turn influence the growth and vitality of the tree host.

We are currently investigating the importance of the genetic component of Norway spruce in determining early formation of ECM symbiosis. The degree of genetic control will be measured as the broad-sense heritability of fungal diversity. Norway spruce trees were sampled for cuttings from Southern Finland last winter. 14-28 rooted cuttings from 55 unrelated trees have been inoculated with the mycelia of five ECM species. In autumn 2009, 10 inoculated and 10 uninoculated cuttings per clone will be sampled for analysis of ECM community. Direct PCR from ECM will be used to enable efficient identification of ECM colonization.

Gremmeniella abietina and artificial pruning induce long-term alterations in foliar chemistry of Scots pine

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The ability of *Gremmeniella abietina* to alter the nutritional status of its host tree was proved experimentally using foliar analyses as main method. The results of three different field experiments describe how severe disease-induced or artificial defoliation, made by pruning of the lowest branches, may induce long lasting (1-10 years) nutritional changes in the foliage of the recovering trees under typical growing conditions in boreal Scots pine (*Pinus* sylvestris L.) stands.

On a general level 40-50% reduction in the live crown length may induce biologically significant changes in the important macro- and micronutrients concentrations, as well as in the C concentrations, of refoliated needles. In a field experiment, foliar concentrations of all the analysed 17 nutrients/elements were affected, to a varying degree, by artificial pruning during the following three years. According to the results presented here, one should be careful when comparing foliar nutrient results alone from injured and healthy conifers, to explain the condition and vitality of trees. Unfortunately, in many studies on defoliated trees, exceptionally high or low needle nutrient concentrations are still often interpreted as one of the causes of tree injury and not, conversely, as the result.

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Heterobasidion spp. fungi host a diverse pool of partitiviruses that show evidence of horizontal transmission between species.

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Mycoviruses with a dsRNA genome occur commonly in filamentous fungi, including *Heterobasidion* spp. In this study we are aimed to elucidate whether the phylogeny of dsRNA viruses found in *Heterobasidion* spp. follows the taxonomy of the host fungi and whether these viruses show strong differentiation according to their geographical origin in Eurasia.

Based on sequence analysis of RNA-dependent RNA polymerase (RdRP) genomic segments, we have found representatives of four major lineages of dsRNA viruses in *Heterobasidion* spp. One of these lineages includes a single observation of a novel virus type that shows some sequence similarity to the *Curvularia* thermal tolerance virus (unclassified dsRNA viruses), while the three remaining clusters can be affiliated with partitiviruses.

RdRP protein similarity between the three *Partitiviridae* clusters varied between 24-39%, while the within-cluster similarity varied from 53% to 99%. One cluster ('1.8kb group') includes a sole virus type isolated from a Chinese *H. insulare* strain and is most closely related to the *Helicobasidium mompa* dsRNA mycovirus. The second ('2.3kb group') cluster includes a sequence type previously described from *H. annosum s.s.* from Sweden (K. Ihrmark) and one dsRNA element we found from *H. parviporum* in Finland (59% protein similarity). The third ('2.0kb group') cluster consists of several virus types related to a sequence previously described from *H. parviporum*, including three dsRNA elements from *H. parviporum* (Finland, Bhutan and China), but also from *H. abietinum* (Greece) and *H. insulare*, the latter showing 99% protein similarity with one of the *H. parviporum* viruses isolated from the same geographical region (Bhutan).

Pairing experiments were conducted to confirm that the dsRNA elements transmit between heterokaryotic isolates of *H. annosum s.l.* and *H. insulare s.l.* Putative capsid protein sequences from the three representative partitivirus groups showed wider sequence diversity than the corresponding RdRP sequences.

In conclusion, our results suggest that there has been recent horizontal transmission of a '2.0kb group' dsRNA element between *H. parviporum* and *H. insulare*. It also seems that the fungal host species have been infected several times by different representatives of *Partitiviridae*, some of the infections postdating the speciation of the host species (this hypothesis is supported by the observation that members of the '2.3kb group' virus pool occur in both of the two North European host species, *H. parviporum* and *H. annosum s.s*).

PCR-DGGE method for detection and identification of *Phytophthora* species from plant material

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One of our aims today is to survey forest and ornamental nurseries to test the occurrence of *Phytophthora* species. Other aim is to find out the commonness and identity of *Phytophthora* species possibly present in imported plant material provided to us by Finnish Food Safety Authority (EVIRA). For these purposes we developed a DNA-based detection method directly from plant material to be used together with traditional isolation and baiting methods.

Phytophthora genus-specific primers are used for PCR-amplification of the ITS1 region, and the amplification products are separated in denaturing gradient gel electrophoresis (DGGE). The specificity of the method was tested with pure cultures of 16 different *Phytophthora*-species; some of them closely related. Different kinds of symptomatic plant materials (artificially inoculated and naturally infected) were used to test the functionality directly from plant hosts. The PCR-DGGE method clearly distinguished 14 of the 16 tested *Phytophthora* species. The detection has also been successful from apple, tomato, rhododendron, strawberry, blueberry, arrowwood, Norway spruce and silver birch.

The advantages of this method are that it should be possible to detect and identify several *Phytophthora* species at the same time from one sample, as well as detecting also previously unknown species. The disadvantages of using this method relate to the sequence diversity in the ITS region, so that species complexes (such as *P. alni* for example) with intraspecific variation in the ITS1 sequence will be difficult to identify.

Gremmeniella infections on seedlings after replanting severely infected pine forest

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During 1999 and 2001 the most severe *Gremmeniella abietina* epidemic ever appeared in Sweden. More than 300 000 ha forest were severely attacked and the forest industry lost milliards of SEK. Big forest areas, with at least 50 000 ha, needed to be clear cut in advance followed by replanting.

For the out-planting experiment pine seedlings were planted on three different locations in Dalarna in Sweden, that were clear-cut in advance due to severe *Gremmeniella* infection. The forest had been clear-cut in 2001 and this study was conducted during 2002-2005. Each site contained one clear cut area and a nearby *Gremmeniella* infected forest. Seedlings were planted on clear-cut areas with and without remaining twigs and branches, at the edge of the clear-cut areas, as well as in the adjacent forest. In total at least 200 seedlings were planted on each area. The areas were replanted every year with new one year old seedlings received from a forest nursery. The disease incident was determined visually the year after plantation and then the infection was confirmed with PCR using *Gremmeniella* specific primers.

For seedling planted at the clear cut areas, the infection decreased from 50 - 90% infected seedlings planted one year after felling to 0 - 55% planted the second year after felling and to 0 -38% for seedling planted three years after felling. After four years there was almost no infection on the clear-cut areas. The variation was big between the sites. Two and three years after felling there were almost no differences between seedlings planted on areas with or without twigs and branches. However seedling planted in the adjacent diseased forest became much more infected then seedlings planted on the clear cut areas. For seedlings planted in the forest one year after felling almost all seedlings became infected. Two and three years after felling they became infected to up to 50 % and even four years after felling 15-40 % of the seedlings became infected. The seedlings planted close to the forest edge were always more infected then the seedlings out on the clear-cut areas but less infected than the seedlings in the forest.
The general conclusion is that it is advisable to wait with replanting with pine seedlings at least two years after felling severely *Gremmeniella* infected stands. Then the risk of infection form twigs and branches left on the clear cut area also is smaller.

Evidence of asexual overwintering of the willow leaf rust fungus *Melampsora larici-epitea*

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Leaf rust, caused by the fungus *Melampsora larici-epitea*, is an economically important disease in biomass willow plantations for renewable energy. *M. larici-epitea* has five spore stages and is heterocious, with willow (*Salix* spp.) as the uredinial, telial and basidial host, and larch (*Larix* spp.) as the spermagonial and aecial host. The complete life cycle, including the sexual phase on larch, has been presumed to be crucial for winter survival of *M. larici-epitea* in northwestern Europe, since evidence of overwintering in the uredinial form has been lacking. Asexual overwintering of urediniospores - were it possible for *M. larici-epitea* – would enable well-adapted genotypes to persist across years and increase to high frequencies, having then important epidemiological consequences.

To investigate the possibility of asexual overwintering, uredospore samples were collected from three locations, one in Northern Ireland, one in Germany and one in Sweden in two consequtive years. To distinguish between genotypes, the DNA fingerprinting technique AFLP was used.

Identical AFLP phenotypes were detected between years in the population in Northern Ireland. In the Swedish and German populations, the same genotypes were not found between years. The results show that *M. larici-epitea* has a mixed reproduction system, with the possibility of overwintering both through sexual reproduction and as clonal lineages. Asexual overwintering is suggested to be of relatively great importance in the Northern Ireland population, while it is infrequent or absent in the Swedish and German populations in this study.

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Endophytic fungi in elms – characterization and interactions with DED fungi

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Endophytic microfungi receive nutrition and shelter from plants, but generally do not cause disease symptoms on their hosts. It has been found that endophytic fungi may positively affect the resistance of plants to biotic and abiotic stress factors. Endophytic fungi have been found in all examined woody plants, but knowledge about the diversity and functional roles of endophytes in trees is still limited.

The goal of our research is to characterize the endophyte diversity in elms (*Ulmus* sp.) and evaluate if certain endophytes can suppress Dutch elm disease (DED), a vascular disease caused by the fungus *Ophiostoma novo-ulmi*. We hypothesize that the endophyte flora may interact with DED fungi through direct competition for the substrate, by excreting bioactive chemicals that affect the pathogen, or by activating the defensive metabolism of the trees. To test these hypotheses, we have isolated endophytic fungi from a set of Spanish field elm (*Ulmus minor*) genotypes with well-characterized resistance traits. Both natural populations that have shown a good resistance to DED and selected clone bank genotypes that represent a gradient of resistance to DED were investigated for the fungal flora in their leaves, bark and xylem.

The results from chemical analyses suggest differences in xylem phenolic profiles between elm genotypes showing different degree of resistance to DED. The isolated fungi were classified to 17 different groups on the basis of colony morphology and colour. Six isolates that could be linked to resistance patterns of the trees were chosen for further studies. The identity of the strains is studied using ITS sequencing and microscopy. Results from primary resource capture tests on MEA show that the aggressive *O. novo-ulmi* is overgrown by some of the isolated fungi or reacts to them by altered colony form or by building a distinct reaction zone in the colony edge closest to the isolate. To further study the mechanisms that may be active in interactions between endophytes and DED fungi, the substrate utilization profiles of the isolates have been analyzed and compared with those of *Ophiostoma* species. The results are discussed with special attention to the potential of endophytes as biocontrol against DED.

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