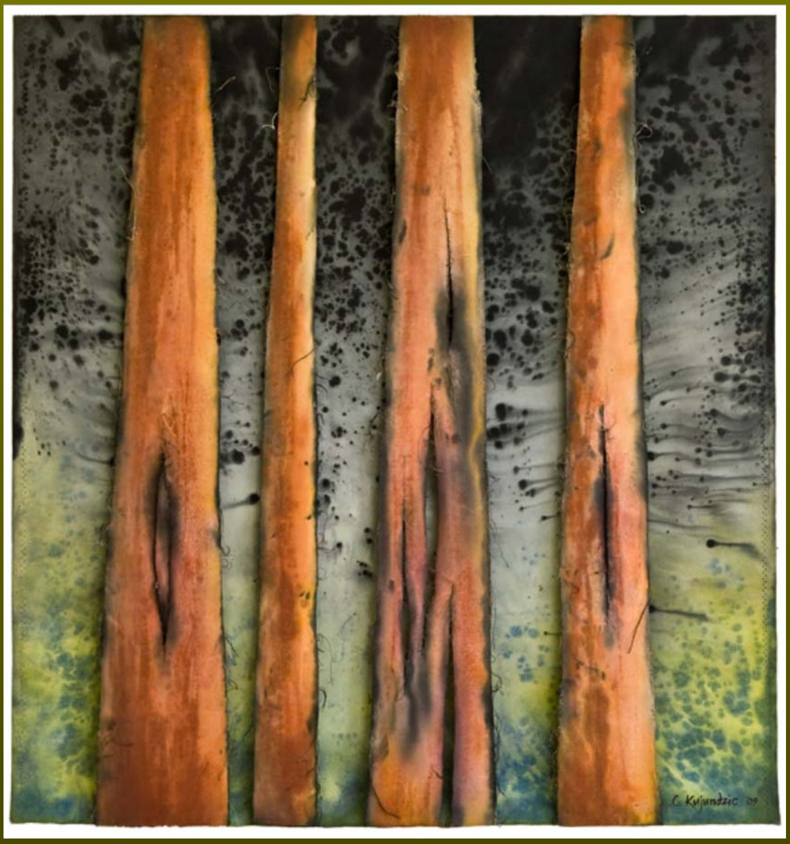
7.02.02 IUFRO WP Foliage, Shoot and Stem Diseases

Global change and forest diseases: new threats new strategies

Montesclaros Monastery, May 23-28th 2011 Cantabria (Spain)



Abstracts book

Edited by: Julio Javier Diez, Pablo Martínez-Álvarez and Carmen Romeralo

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IUFRO 2011 WORKING PARTY 7.02.02

Foliage, shoot and stem diseases of forest trees

GLOBAL CHANGE AND FOREST DISEASES: NEW THREATS, NEW STRATEGIES

CONFERENCE PROGRAMME
ABSTRACTS
LIST OF PARTICIPANTS

Montesclaros Monastery, Cantabria, Spain

23rd to 28th of May 2011

IUFRO Division 7 - Forest Health

7.02.02 - Foliage, shoot and stem diseases

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CONFERENCE PROGRAMME

Sunday 22nd May

Arrival of the participants

Monday 23rd May

7.00-8.00	Registration of the participants in the Monastery
8.15-9.00	Breakfast
9.15-11.00	Transportation to Palencia Campus (University of Valladolid)
11.00-13.00	Opening ceremony
13.00-14.00	**Inaugural conference** by M. J. Wingfield. **Global Change and Tree Diseases: New Threats and New Strategies**
14.00-15.00	Lunch
15.30-17.00	City tour
17.00-17.30	Self introduction of the participants
Session 1: Scleroderris (*Gremmeniella*) canker Moderator: Antti Uotila	
17.30-17.50	L. Botella, T. T. Tuomivirta, J. Hantula and J. J. Diez. **Mycoviruses are also Present in the Spanish Population of *Gremmeniella abietina***
17.50-18.10	A. G. Aday, A. Lehtijarvi and H. T. Doğmuş-Lehtijarvi. **Presence of Double-Stranded RNA in some Turkish *Diplodia pinea* and *Gremmeniella abietina* Isolates**
18.10-18.30	C. Romeralo, L. Botella, O. Santamaría and J. J. Diez. **Effects of Temperature, pH and Osmotic Potential on in Vitro Mycelial Growth of *Gremmeniella abietina* Isolates Infected by Mitoviruses**
18.30-19.00	Coffee break
19.00-19.20	E. Stenström, J. Oliva, L. G. Wichmann, K. Wahlström, M. Jonsson, I. Drobyshev and J. Stenlid. ***Gremmeniella* Epidemic in Sweden in 1999 and 2001 - Recovering of the Forest**
19.20-19.40	H. T. Doğmuş-Lehtijarvi, F. Oskay, M. Karadeniz and A. Lehtijarvi. **Susceptibility of *Pinus nigra* and *Cedrus libani* to Turkish *Gremmeniella abietina* Isolates**
19.40-20.00	E. Smerlis and G. Laflamme. **Pathogenicity Trials with *Gremmeniella* Fungi Collected on Conifers in Canada**
20.15-21.00	Reception at Palencia Council
21.00-22.30	Return to the Monastery

Tuesday 24th May

8.15-9.00	Breakfast
Session 2: *Diplodia* shoot blight and Pitch canker Moderator: Oscar Santamaría	
9.15-9.35	B. W. Oblinger, D. R. Smith and G. R. Stanosz. **Red Pine Logging Debris as a Potential Source of Inoculum of *Diplodia* Shoot Blight Pathogens**
9.35-9.55	D. R. Smith and G. R. Stanosz. **Storage Conditions Influence Cultural Detection of the Shoot Blight Pathogen *Diplodia pinea* on or in Asymptomatic Red Pine Nursery Seedlings**
9.55-10.15	N. Luchi, V. Mancini, M. Feducci, A. Santini and P. Capretti. **Transmission of *Diplodia pinea* Via the New Invasive Insect *Leptoglossus occidentalis***
10.15-10.35	D. Bezos, J. M. Lomba, P. Martínez-Álvarez, M. M. Fernández and J. J. Diez. **Effects of Pruning on Pitch Canker Disease in *Pinus radiata* Plantations**
10.35-10.55	P. Martínez-Álvarez, J. Blanco, M. de Vallejo, F. M. Alves-Santos and J. J. Diez. **Susceptibility of several conifers to pitch canker disease**
10.55-11.15	C. Agustí-Brisach, M. Berbegal, A. Pérez-Sierra, J. Armengol and J. García-Jiménez. **Hot water treatment to reduce *Fusarium circinatum* contamination on *Pinus radiata* seeds**
11.15-11.45	Coffee break
Session 3: *Dothistroma* pine needle cast Moderator: Paolo Capretti	
11.45-12.05	J. Janoušek, R. McDougal, M. Mullet, L. Jankovský, A. Brown and R. E. Bradshaw. **Quantification of *Dothistroma septosporum* Spores by Real-Time PCR**
12.05-12.25	M. Dvořák and L. Jankovský. ***Dothistroma septosporum*: Incidence of Spore Production and Weather Condition**
12.25-12.45	M. Vuorinen. **The Occurrence of *Dothistroma septosporum* in Different Types of Forests in Finland**
13.00-14.00	Lunch
Session 4: Foliage and dieback diseases Moderator: Jarkko Hantula	
14.30-14.50	V. Talgø and A. Stensvand. **Foliage Diseases on True Fir in Norway**
14.50-15.10	M. M. Müller and T. Sieber. **Does Long Distance Gene Flow Occur Between Subpopulations of *Lophodermium piceae*, the Most Common Needle Endophyte of Norway Spruce?**
15.10-15.30	G. Laflamme, C. Côté and L. Innes. **White Pine Needle Diseases in Eastern Canada**
15.30-15.50	F. Oskay, E. Halmschlager, A. Lehtijarvi and H. T. Doğmuş-Lehtijarvi. ***Cedrus libani*, a New Host for *Herpotrichia juniperi***
15.50-16.10	T. Hsiang, A. Darbyson, P. Goodwin, A. Cortes-Barco and B. Nash. **Enhancing Systemic Resistance of Maple Against Tar Spot Disease**
16.10-16.30	K. Davydenko, S. Bengtsson, J. Stenlid and R. Vasaitis. **Fungi in Shoots and Foliage of *Fraxinus excelsior* and *F. angustifolia* in Eastern Ukraine**

16.30-16.50	T. Kirisits and C. Freinschlag. **Ash Dieback in a Seed Plantation in Austria**
16.50-17.30	Coffee break
Session 5: Bark diseases Moderator: Gaston Laflamme	
17.30-17.50	I. A Munck and P. D. Manion. **Impact of Beech Bark Disease on the Sustainability of American Beech in New York**
17.50-18.10	A. Lilja, A. Rytkönen and J. Hantula. ***Neonectria* sp., a New Pathogen Causing Cankers on Norway Spruce?**
18.10-18.30	V. Talgø, M. B. Brurberg and A. Stensvand. ***Neonectria*-Canker on Trees in Norway**
18.30-18.50	A. Lehtijarvi, F. Oskay, A. G. Aday and H. T. Doğmuş-Lehtijarvi· **Pathogenicity of some Fungi Isolated from Cankers on *Cupressus sempervirens* var. *horizontalis* in Turkey**
18.50-19.10	J. Kaitera and R. Hiltunen. **New Alternate Hosts for *Cronartium* spp. in Finland**
19.10-19.30	J. Martín-García, M. M. Müller and J. J. Diez. **Differences in twig endophyte assemblages between native black poplar (*Populus nigra*) and a cultivated hybrid poplar (*Populus x euramericana*)**
19.30-20.30	C. Kujundzic **Message from the beetle (art exhibition)**
20.30-21.30	Dinner

Wednesday 25th May

Tree Disease Field Trip 1:
Fusarium circinatum on *Pinus radiata*, and *Mycosphaerella* sp. on *Eucalyptus globulus* plantations.

Social activities: Monastery tour

Thursday 26th May

8.15-9.00	Breakfast
Session 6: Other diseases I Moderator: Tugba Doğmuş-Lehtijärvi	
9.00-9.20	M. Hultberg, K. Blumenstein and J. Witzell. **The Potential of Soil Bacteria and their Biosurfactants to Suppress *Phytophthora* Diseases of Forest Trees**
9.20-9.40	S. Akilli, Ç. Ulubas Serçe, Y. Z. Katircioglu and S. Maden. ***Phytophthora* Diseases of Chestnut Trees in Black Sea Region of Turkey**
9.40-10.00	M. M. Haque and J. J. Diez **Susceptibility Assessment of Common Alder Seedlings to *Phytophthora alni* and Other *Phytophthora* Species**
10.00-10.20	G. Sanchez, J. J. Tuset, C. Hinarejos, J. L. Mira and M. Prieto. **Research on Oak Decline Disease in Spain**
10.20-10.40	J. Hantula, L. Hamberg, H. Vartiamäki, K. Korhonen and A. Uotila. **On the Use of *Chondrostereum purpureum* in Controlling Hardwood Sprouting**
10.40-11.00	A. Uotila and J. Levula. **The Soil Temperatures During Prescribed Burning and the Occurrence of *Rhizina undulata* Fr.**
11.00-11.30	Coffee break
11.30-11.50	S. Green, B. E. Laue, H. Steele, G. A. MacAskill and P. M. Sharp. **Using Genomics to Gain Insights into the Evolution and Biology of *Pseudomonas syringae* pv. *aesculi* on European Horse Chestnut**
11.50-12.10	T. Hsiang. **Sequencing and Assembly of a Fungal Genome**
12.10-12.30	M. Feducci, N. Luchi, V. Mancini and P. Capretti. **Phytosanitary Conditions of *Quercus cerris* in Tuscany Evaluated by Monitoring, GIS Applications and Molecular Techniques**
12.30-12.50	M. Vivas, J. A. Martín and A. Solla. **Evaluating Methyl Jasmonate for Induction of Resistance Against *Fusarium oxysporum*, *F. circinatum* and *Ophiostoma novo-ulmi***
13.00-14.00	Lunch
Session 6: Other diseases II Moderator: Mike Wingfield	
14.30-14.50	G. Álvarez Baz, I. Etxebeste, G. Pérez, A. Martín, E. Sánchez-Husillos, D. Hall and J. A. Pajares. **Semiochemicals for Monitoring and Control of the Pine Wood Nematode Vector *Monochamus galloprovincialis* (Coleoptera: Cerambycidae)**

14.50-15.10	T. Misik, I. Kárász and B. Tóthmérész. **Changes of the Structure in a Turkey-Oak Forest after a Tree Decline in North-Hungary**
15.10-15.30	K. Kuroda, K. Osumi and H. Oku. **How to Recover the Health of Secondary Forest "Satoyama" Declining by the Japanese Oak Wilt**
15.30-15.50	R. Sturrock. **Climate Change and Forest Diseases: Using Today's Knowledge to Address Future Challenges**
16.00-17.00	Seminar: **Global Change and Forest Diseases**
17.00-17.30	Coffee break
17.30-18.30	Poster session
18.30-19.30	Business meeting
21.00	Social event

Friday 27th May

Tree Disease Field Trip 2:
Pinus pinaster decline and *Gremmeniella abietina* on *Pinus halepensis* stands.

Saturday 28th May

Depart of the participants.

Poster session
G. Balmelli, S. Simeto, N. Altier, V. Marroni and J. J. Diez. **Effects of Leaf Spotting Caused by Mycosphaerella Leaf Disease and Eucalyptus Rust on *Eucalyptus globulus* in Uruguay**
K. Černý, V. Strnadová and N. Filipová. **Can Global Warming Affect the Survival and Impact of *P. alni* subsp. *alni*?**
C. Colinas and V. J Monleon. **An Assessment of the Early Effects of Climate Change on Forest Health**
H. T. Doğmuş-Lehtijarvi, A. G. Aday, and A. Lehtijarvi, **Powdery Mildew Fungi on some Deciduous Tree Species in Turkey**
S. Dorji, E. Donaubauer, M. J. Wingfield, D. B. Chhetri and T. Kirisits. **Himalayan Dwarf Mistletoe (*Arceuthobium minutissimum*) and the Leafy Mistletoe *Taxillus kaempferi* on Blue Pine (*Pinus wallichiana*) in Bhutan**
M. Keßler, T. L. Cech, C. Tomiczek and E. Halmschlager. ***Mycosphaerella dearnessii* M. E. Barr (Brown-Spot Needle Blight of Pine) in Austria**
M. Keßler, T. L. Cech, M. Brandstetter and T. Kirisits. **Dieback of Ash (*Fraxinus excelsior* and *Fraxinus angustifolia*) in Eastern Austria: Disease Development on Monitoring Plots from 2007 to 2010**
T. Kirisits and T. L. Cech. **Alien Pathogens of Forest Trees in Austria**
T. Kirisits, M. Matlakova and E. Halmschlager. **Ash Dieback in Forest Nurseries in Austria**
T. Kirisits, H. Konrad, M. J. Wingfield and D. B. Chhetri. **Ophiostomatoid Fungi Associated with the Eastern Himalayan Spruce Bark Beetle (*Ips schmutzenhoferi*) in Bhutan: Species Assemblage and Phytopathogenicity**
A. Koltay, I. Szabó and G. Janik. ***Chalara fraxinea* Incidence in Hungarian Ash (*Fraxinus excelsior*) Forests**
J. Kovács, I. Szabó and F. Lakatos. **Phytophthora-Infection in a Sweet Chestnut (*Castanea sativa*) Orchard in Transdanubia, Hungary**
K. Kräutler and T. Kirisits. **The Ash Dieback Pathogen *Hymenoscyphus pseudoalbidus* is Associated with Leaf Symptoms on Ash Species (*Fraxinus* spp.)**
A. Lehtijarvi and H. T. Doğmuş-Lehtijarvi. **Seasonal Variation in the Infection Level of *Cedrus libani* Needles by *Ploioderma cedri***
T. Májek and V. Tomešová. **Red Band Needle Blight – Molecular Screening of the Czech Republic**
J. A. Martín, J. Witzell, K. Blumenstein and L. Gil. **Antagonistic Effect and Reduction of *Ulmus minor* Symptoms to *Ophiostoma novo-ulmi* by Elm Endophytes**
P. Martínez-Álvarez, J. Martín-García and J. J. Diez. **Endophyte communities associated with northern Spain forests: influence of environmental variables**
I. A Munck and R. L. Lilja. **Monitoring Damage from Foliage, Shoot and Stem Diseases in New England and New York**
L. Nagy and I. Szabó. **Epidemic and Pathogenicity of *Chalara fraxinea* Causing Ash Dieback in Hungary**
C. Prieto-Recio, F. Bravo and J. J. Diez. **REINFFORCE (REsource INFrastructure for monitoring and adapting European Atlantic FORests under Changing climatE)"Establishment a Network of Arboretums and Demonstration Sites to Assess Damages Caused by Biotic and Abiotic Factors"**
E. Quintana, Y. Serrano, N. Mesanza, M. Elvira-Recuenco, R. Raposo and E. Iturritxa. **Evaluation of Genetic Resistance to *Fusarium circinatum* in *Pinus* Species**

A. Rytkönen, A. Lilja, M. Soukainen, P. Parikka, S. Werres, M. Poteri and J. Hantula. **Investigations on *P. plurivora and P. pini* in Finland**
O. Santamaría, C. Romeralo, L. Tejerina and J. J. Diez O. **Interaction Between *Gremmeniella abietina* and Several Fungal Endophytes**
A. V. Sanz-Ros, F. Valladares and J. J. Diez. **Factors Influencing Canopy Loss in Pine Stands Infected by *Gremmeniela abietina* in Northern Spain**
Y. Serrano, E. Quintana, M. Elvira-Recuenco, E. Iturritxa and R. Raposo. **Effect of Temperature on Survival of *Fusarium circinatum***
M. C. Silva, H. N. Machado, L. Neves, C. Valente and A. J. L. Phillips. **Distribution of *Mycosphaerella* Leaf Disease on Eucalyptus in Portugal**
D. R. Smith and G. R. Stanosz. **Cultural Detection of *Diplodia* Shoot Blight Pathogens from Red Pine and Jack Pine Seeds**
G. R. Stanosz and D. R. Smith. **Expansion in the Known Geographic Distribution and Host Range of the Shoot Blight Pathogen *Sirococcus tsugae***
V. Talgø, J. I. S. Perminow, A. Sletten, M. B. Brurberg, M. L. Herrero, G. M. Strømeng, and A. Stensvand. **Diseases on Horse Chestnut in Norway**
V. Tomešová, J. Janoušek, L. Jankovský, D. Palovčíková, M. Dvořák and T. Májek. ***Dothistroma septosporum* and *Lecanosticta acicola* in the Czech Republic: Current Situation and Inoculation Tests**
P. Zamora, A. B. Martín, R. San Martín and J. J. Diez. **Control of Chestnut Canker with Hypovirulent Strains of *Cryphonectria parasitica* in Castilla y León (Spain)**

ABSTRACTS

ABSTRACTS

Oral presentations

Inaugural Conference

Global Change and Tree Diseases: New Threats and New Strategies

M. J. Wingfield

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Robert Hartig's classic first forest pathology text book "Lehrbuch der Baumkrankheiten" was published in 1882, about 130 years ago. It is interesting that this work, and indeed forest pathology as a discipline, emerged at more or less the same time as some of the most notable examples (white pine blister rust, chestnut blight, Dutch elm disease) of epidemic tree diseases caused by accidentally introduced pathogens. Research in subsequent years has led to a broad understanding of the biology, ecology and management of tree pathogens. It is well established that tree pathogens are regularly being moved to new environments and the field of invasion biology has emerged as an important component of forest pathology. New tools, particularly those linked to DNA-based technologies have led to fascinating new discoveries relating to tree pathogens. Exciting and important developments include the ability to recognise cryptic pathogen species that were previously not detectable. This has also made it possible to detect important and unexpected tree pathogen host shifts. Furthermore, tree endophytes have emerged as important in tree health, both as pathogens and possibly relating to the protection of trees against the onslaughts of pests and diseases. The growing numbers of pathogen and tree genomes available for study, new generation DNA sequencing and metagenomics will influence the future of forest pathology dramatically. Against the backdrop of these powerful new tools to study and better understand tree diseases, there will be many threats to tree health in years to come. Examples of negative impacts of global climate change on tree health are emerging and this is a trend that will most likely continue. The movement of trees to new environments will lead to increasing numbers of "new encounter" diseases. Likewise, growing global trade and tourism will make it increasingly difficult to avoid the movement of tree pathogens to new environments, where they come into contact with susceptible hosts.

Session 1: Scleroderris (*Gremmeniella*) canker

Mycoviruses are also Present in the Spanish Population of *Gremmeniella abietina*

L. Botella[1], T. T. Tuomivirta[2], J. Hantula[2] and J. J. Diez[1]

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Gremmeniella abietina var. *abietina* has commonly been pointed out as a species complex, which includes different races and biotypes. Among them, the Spanish population seems to be a unique differentiated population derived from Biotype A. Furthermore, *G. abietina* is known to harbour infections and co-infections of putative mycoviruses belonging to different families. In particular, *G. abietina* type A harbours putative members of families *Totiviridae* and *Partitiviridae* but also *Narnaviridae*, with members of genus *Mitovirus.* In case of *G. abietina* type B, a novel putative endornavirus has been identified and occurrence of mitoviruses molecules have been analysed but no published. Different types of *G. abietina* seem to host a divergent virus community. So, ninety-one isolates of the Spanish *Gremmeniella abietina* were analyzed to check out whether their genome harboured viral dsRNA particles. Thus, the 86.8 % of Spanish isolates presented at least one dsRNA particle, which is a significant frequency. Overall, 8 dsRNA banding patterns were detected, suggesting the occurrence of putative members of different virus genera as *Partitivirus*, *Mitovirus* and *Totivirus*. These results were supported with sequencing and compared in Blastx protein database.
As genetic variability of mycoviruses in *G. abietina* seems to be related to the own fungus genetic structure. The question was compulsory: Do Spanish population of *G. abietina* has its own virus population? And are they more related to mycoviruses of Biotype A or in B? Preliminary results suggest that even though the genetic diversity structure of the fungus is very low, the variability of its viruses is high enough and they seems to be related to both A and B biotypes.

Presence of Double-Stranded RNA in some Turkish *Diplodia pinea* and *Gremmeniella abietina* Isolates

A. G. Aday[1], A. Lehtijarvi[2] and H. T. Doğmuş-Lehtijarvi[2]

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Diplodia pinea and *Gremmeniella abietina* are common pathogens causing shoot blight and dieback of pine all over the world. *D. pinea* is one of the main causal agents of shoot blight of Calabrian pines in the Mediterranean countries including Turkey. *G. abietina* has been recently observed on saplings and seedlings of *Pinus nigra*, which remain under snow cover during winter dormancy in Dedegül Mountain in the Mediterranean Region of Turkey.

The presence of viruses in fungi has been known for many years. An accumulating number of cloned and sequenced viral genomes has enabled us to detect virus in increasing number of fungal species in the recent years. *D. pinea* and *G. abietina* are known to contain members of the virus families Narnaviridae, Totiviridae and Partitiviridae, which can infect single fungal isolates. Viral dispersal in fungi mainly occurs via anastomosis. Some *Diplodia* and *G. abietina* isolates have different characteristics, such as reduced virulence and growth rate, lack of pigmentation, altered colony morphology, and reduction in conidial production due to presence of viral particles.

In this study, eighteen *D. pinea* and six *G. abietina* isolates were investigated for the presence of dsRNA. Double-stranded RNA was isolated using a commercial RNA extraction kit and visualized in agarose gel electrophoresis. Isolates containing dsRNA were also investigated for their *in vitro* growth rate and ability to produce conidia. Three (50%) *G. abietina* and ten (56%) *D. pinea* isolates contained dsRNA that had an approximate molecular size of 1.6 kb

Effects of Temperature, pH and Osmotic Potential on *in Vitro* Mycelial Growth of *Gremmeniella abietina* Isolates Infected by Mitoviruses

C. Romeralo[1], L. Botella [1], O. Santamaría [2] and J. J. Diez [1]

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Mitoviruses have been found in several forest pathogens (e.g. *Gremmeniella abietina*) and because they may reduce virulence of the host fungi their use is being studied for biocontrol purposes. A preliminary study was carried out to test the effect of temperature (5 º, 15 º, 25 º and 35 º C), pH (4, 5, 7 and 9) and osmotic potential ([KCl] of 250, 500, 750 and 1000 mM) on mycelial growth of seven *G. abietina* isolates under laboratory conditions. Four of the isolates hosted mitoviruses and three of them did not. During the experiment, mycelial growth was measured every week for a period of 8 weeks. Highest colony sizes were observed in the Petri dishes with pH 4 and 5, temperature of 15 º C and 1000mM of KCl, and lowest sizes in the ones placed at 35 º C. No differences were observed among isolates in experiments developed at 5 º and 25 º C and pH of 9. However, Petri dishes placed at 15 º C presented differences on mycelial growth if isolates were grouped in mitovirus and not mitovirus presence. Colony areas measured in pH of 4, 5 7 and 750mM KCl treatments presented differences among isolates when analyzed altogether. Mycelial growths of isolates with mitovirus were higher than the ones without mitoviruses at 15 º C (p=0.0188) and 1000mM KCl (p=0.0001). On the contrary, they were lower in dishes with 250mM (p=0.0086) and 750mM (p<0.0001) KCl. The significance of the occurrence of mitovirus on the mycelial growth of these isolates is discussed.

Gremmeniella Epidemic in Sweden in 1999 and 2001 - Recovering of the Forest

E. Stenström[1], J. Oliva[1], L. G. Wichmann[1], K. Wahlström[1], M. Jonsson[1], I. Drobyshev[2] and J. Stenlid[1]

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During 1999 and 2001, the most severe *Gremmeniella abietina* epidemics ever appeared in Sweden. More than 500,000 ha forest were severely attacked and the forest industry lost milliards of Euros. In order to follow the development of the forest after one or two *Gremmeniella* infections, we studied seven experimental sites established in the most affected areas in middle of Sweden. In total, we followed the defoliation and growth of 360 trees exposed to two epidemics and of 250 trees exposed to one epidemic. When the experiment started in 2000 and 2001, trees were chosen according to different defoliation: healthy (<20% defoliation), medium (60-70%) or severely defoliated (80-90%). The number of epidemics affected the survival of trees in the medium defoliation class, but not in the severely and healthy classes. When subjected to two epidemics, survival after 10 years of medium defoliated trees was almost similar to that of severely defoliated trees (40% vs. 25%, respectively). When only attacked once, both medium and severely defoliated trees showed a higher survival (65% vs. 35%). After one epidemic, surviving trees presented a lower defoliation (25%) than when subjected to two epidemics (50%) i.e. less defoliated trees survived. We also observed different patterns amongst surviving trees subjected to one or two epidemics. Severely defoliated trees subjected to one epidemic recovered the growth at a similar rate than trees with medium defoliation. Severely infected trees subjected to two epidemics recovered the growth at a lower rate than trees with medium defoliation. Recurrent epidemics severely diminish the capacity of survival and recovery from *G. abietina* attacks. Knowledge on preceding attacks may be used to optimize tree removal after the epidemics. Trees shall be removed based upon different defoliation thresholds depending on the previous history of the stand.

Susceptibility of *Pinus nigra* and *Cedrus libani* to Turkish *Gremmeniella abietina* Isolates

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Virulence of Turkish *Gremmeniella abietina* isolates was investigated in a field experiment. Five isolates obtained from dead branches of *Pinus nigra* subsp. *pallasiana* and *Pinus sylvestris* in high altitude mountainous areas in the Black Sea region and the Lakes District were used. The lower branches of 15-20 year-old *P. nigra* and *C. libani* in a plantation site at 1050m a.s.l. in Isparta, were inoculated at 1-2-month intervals during September-January. Each isolate was inoculated into one branch per tree and repeated ten times on both tree species at each inoculation date; totally eighty trees and four hundred-eighty branches were inoculated. The branches were harvested in the end of February, after approximately 170, 110, 60 and 30 days of incubation and the length of necrosis in the inner bark measured (inoculation wound excluded). The mean length of necrosis on *P.nigra* and *C libani* were 10.6±0.8 and 3.8±0.2 mm, respectively. In general, differences in the mean length of necrosis between the isolates were small. Nevertheless, there were significant differences between the isolates on *P. nigra* in November and January inoculations, and on *C. libani* at all four inoculation dates. The mean necrosis length for all isolates was the highest ($p<0.01$) in December inoculations for both *P. nigra* (22.0±1.9) and *C. libani* (5.6±0.7). There was no difference between the September and January inoculations on *P. nigra,* despite the almost six-fold difference in incubation period. On *C. libani,* in contrast, the shortest necroses were found in January inoculations ($p<0.05$). During the December inoculations, the trees were most likely in winter dormancy, which would explain the large lesions.

Pathogenicity Trials with *Gremmeniella* Fungi Collected on Conifers in Canada

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Gremmeniella abietina var. *balsamea* isolated from balsam fir (*Abies balsamea*) and spruces (*Picea* spp.) was tested for pathogenicity on different conifer hosts including *A. balsamea* and *Picea* spp. Pathogenicity of the fungus was positive on balsam fir only. This pathogen could not colonize other conifers, not even spruces which are hosts included in the taxonomic entity *G. abietina* var. *balsamea*. Also, inoculation trials with isolates from spruces and pines on several conifer species are specific to their respective hosts. These results raise questions on the taxonomic status of the two pathogens classified as var. *balsamea*. We believe that both pathogens on spruce and balsam fir should be promoted to the species level for two reasons: 1) isolates from balsam fir, spruces and pine are specific to their hosts, and 2) they have a colour in pure culture that is characteristic of each three groups of isolates. The species *G. laricina* is morphologically very different from all other known species of *Gremmeniella*. All *Gremmeniella* native to North America cause damage only on shoots in the snow.

Session 2: *Diplodia* shoot blight and Pitch canker

Red Pine Logging Debris as a Potential Source of Inoculum of *Diplodia* Shoot Blight Pathogens

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Following observation of a high incidence of *Diplodia* shoot blight on recently planted red pine (*Pinus resinosa*) seedlings growing where mature red pine stands previously had been clearcut, the potential of logging debris as a source of inoculum of *Diplodia* pathogens was investigated. Cones, bark, needles, stems from shoots bearing needles, and stems from shoots not bearing needles (both suspended above the soil and in soil contact) were collected from debris left at sites that were previously clearcut. Conidia were extracted in water, quantified, tested for germinability, and *Diplodia* species were identified from samples using a PCR assay. Conidia of *Diplodia* species were detected from all study sites. Repeated sampling of the same sites at 6, 12, and 18 months postharvest revealed an initial increase in numbers of conidia. Although fewer conidia were obtained from debris collected from additional sites at greater intervals since harvest (and fewer of these germinated), all substrates yielded many germinable conidia even 5 years postharvest. The type of host substrate from which conidia were extracted had an effect on the number of conidia quantified and the percentage of conidia that germinated. Also, more conidia were obtained and a greater percentage germinated from debris that was suspended above soil at the time of collection than from debris in soil contact. Because red pine seedlings are commonly planted in close proximity to logging debris on clearcut sites and germinable conidia were abundant, debris could be a potential persistent source of inoculum for *Diplodia* shoot blight pathogens to planted seedlings. The results of this study should prompt further consideration by land managers of the potential forest health risks, in addition to benefits, that may be associated with logging debris.

Storage Conditions Influence Cultural Detection of the Shoot Blight Pathogen *Diplodia pinea* on or in Asymptomatic Red Pine Nursery Seedlings

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The pine shoot blight and canker pathogen *Diplodia pinea* has been shown to persist on or in asymptomatic red pine nursery seedlings, with later potential to rapidly proliferate after outplanting to cause disease, including seedling mortality. After lifting from nursery beds, seedlings are routinely kept in cold storage at nurseries, but during and after shipment to customers may be maintained without refrigeration for several days prior to planting. The potential for both the duration and temperature of storage to influence the frequency of cultural detection of *D. pinea* from asymptomatic red pine seedlings was investigated. In the first two experiments, surface-disinfested stem segments from seedlings were culturally assayed for *D. pinea*: shortly after lifting in spring; after 3 weeks of cold storage (approximately 4 degrees C in experiment 1) or 4 weeks of cold storage (approximately 8 degrees C in experiment 2); or after 3 weeks of cold storage followed by 1 week of storage at approximately 24 degrees C in both experiments). Probably due to implementation of a program of scrupulous sanitation and application of preventative fungicidal sprays at the nursery, *D. pinea* was infrequently detected, and no effects of storage were apparent. In two additional experiments, seedlings were inoculated with a suspension of *D. pinea* conidia and then similarly assayed: after 3 weeks of cold storage (approximately 4 degrees C in experiment 3) or 4 weeks cold storage (approximately 8 degrees C in experiment 4); or after 3 weeks of cold storage followed by 1 week of storage at approximately 24 degrees C in both experiments. In experiments 3 and 4, in which the pathogen was initially present due to inoculation, frequency of detection of the pathogen was greater after longer storage and after storage at a warmer temperature. This indicates that the association of the pathogen with seedlings may be affected by storage conditions. Thus, when inoculum is present, minimization of the duration of storage and maintenance of cold temperatures during storage may inhibit persistence of *D. pinea* on or in seedlings, and help to reduce later seedling mortality.

Transmission of *Diplodia pinea* Via the New Invasive Insect *Leptoglossus occidentalis*

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During the last years global changing showed its impact on forest ecosystems. In this context the presence of new invasive species, introduced from different geographical areas, may have stronger effects on native species leading to changes on tripartite interactions among plant-pathogen-insect associations.
Since 1999 in Italy a new insect species, *Leptoglossus occidentalis*, originated from North America, has been introduced. This insect which is able to causes several damages on cones of conifer trees, has spread on *Pinus pinea* (Italian Stone pine) plantations. This pine species is also one of the hosts of a native fungus (*Diplodia pinea*) which is becoming an increasing threat because of stresses due to global warming.
Since both insect and fungus have been found living in the cones of the same host, a possible interaction between these two organisms has been hypothesized and a molecular method developed by using real-time PCR (TaqMan™ chemistry) was used to detect and quantify the fungal presence on the insect body.
The aim of this study, supported by the project PINITALY (MiPAAF DM 256/7303/2007), was to ascertain whether *L. occidentalis* could act as possible vector of *D. pinea* on *P. pinea* cones. For this purpose groups of individuals insects collected in the forest but also raised in laboratory were analyzed. In the lab insects were processed after: i) artificial contamination with a conidial suspension of *D. pinea*; ii) walking in captivity on pine cones infected with *D. pinea.* Samples not treated were used as negative control.
The conidial contamination by soaking insect body reproduced a possible source of conidia after rainfall, while the walking on infected cones simulated another possible type of insect contamination.
Molecular analysis after real-time PCR showed the presence of *D. pinea* DNA on insects, either in the case of those collected from forest and also from laboratory, showing significant differences among the different thesis.
This study, confirmed the hypothesis of a possible role of *L. occidentalis* as vector of *D. pinea*. The use of rapid and sensitive molecular tools leads to detect a fungal pathogen in a DNA extracted from insect body, revealing the association between the native fungal pathogen and the invasive insect species.

Effects of Pruning on Pitch Canker Disease in *Pinus radiata* Plantations

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Fusarium circinatum (Niremberg and O´Donnell 1998) is the causal agent of Pitch Canker Disease (PCD) in *Pinus* species, causing necrosis and deformation in trunk as well as dieback. The disease appeared recently in northern Spain associated to *Pinus* spp. seedlings at forest nurseries, and *P. radiata* plantations in the forest. The aim of the present study was to evaluate the effect of pruning on PCD in *Pinus radiata* plantations in Cantabria, so the study was carried out on 50 *Pinus radiata* plots (pruned and unpruned) distributed along this region. Symptoms of PCD were evaluated in 25 trees in each plot following the ICP Forest methodology and were related with dendrometric factors including pruning. A significant relationship was found between pruning and the number of cankers per tree concluding that management affects PCD severity.

Susceptibility of Several Conifers to Pitch Canker Disease

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Fusarium circinatum is the causal agent of the Pitch Canker Disease, which was first detected in Spain in 2004. Not only *Pinus radiata* and *P. pinaster* seedlings in nurseries were affected but also *P. radiata* plantations. Thus, the pathogen has spread out over many pine forests of northern Spain producing substantial economical losses. Consequently, a resistant and viable pine species is needed to be found as alternative. The susceptibility of several conifer species was tested in laboratory conditions measuring the germination of seeds and mortality of the emerged seedlings. In adition, a complementary field experiment was also established to evaluate natural infection of different conifers. *Chamaecyparis lawsoniana* and *Sequoiadendron giganteum* obtained the best results in the assay developed in the laboratory. On the contrary, all *P. radiata* seedlings died, confirming that it is the most susceptible species. In relation to the field experiment, *P. radiata* and *P. pinaster* showed the highest growth. Finally, natural infection did not occurred during the first two years after plantation.

Hot water treatment to reduce *Fusarium circinatum* contamination on *Pinus radiata* seeds

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Fusarium circinatum is the causal agent of pitch canker on *Pinus* spp. and *Pseudotsuga menziessi*. The use of pathogen-free seeds is the most important means to prevent infections in nurseries and long-distance spread. In this work, different temperature-time combinations of hot water treatment (HWT) were evaluated to reduce *F. circinatum* contamination of *Pinus radiata* seeds without compromising seed quality. Specific objectives of the research were: (i) to evaluate the sensitivity of *F. circinatum* isolates to HWT *in vitro*, (ii) to evaluate *P. radiata* seed germination after HWT, and (iii) to assess the effect of HWT on naturally infected seeds of *P. radiata*.

Four *F. circinatum* isolates (two of each mating type) were used to evaluate mycelial growth and conidial germination after treatments at 47, 48, 49, 50, 51, 52, 53 and 54 ºC for 30 or 45 minutes. Results showed a significant reduction of mycelial growth and conidial germination associated with an increase of treatment temperature and duration time. Mycelial growth was not observed at temperatures above 50 and 51 ºC for isolates in *MAT*-2 and *MAT*-1, respectively. Differences were observed on conidial germination among the isolates depending on the mating type: at 49 ºC no conidial germination was observed for *MAT*-2 isolates, however, *MAT*-1 isolates tolerated temperatures up to 52-53 ºC although percentages of germination were very low.

*P. radiata s*eed germination was evaluated after treatments at 50, 51, 52, 53 and 54 ºC for 30 or 45 min. Percentage of germination in non-treated seeds was 90% and, in treated seeds, percentage of germination decreased significantly with increasing temperature and time. Percentage of germination values ranged from 80% at 50ºC for 30 or 45 min to 55-40% at 54ºC for 30 and 45 min, respectively.

Reduction of *F. circinatum* contamination was tested on naturally infected *P. radiata* seeds after treatments at 50, 51, 52, 53 and 54 ºC for 30 or 45 min. HWT effects were evaluated by assessing the percentage of infected seeds plated onto Komada medium. Percentage of infection of non-treated seeds in Komada was above 70% while only 1% of infected seeds were detected after 50ºC-30 min, 50ºC-45 min and 52ºC-45 min treatments. Results obtained indicate that HWT reduce *F. circinatum* contamination of *P. radiata* seeds.

Session 3: *Dothistroma* pine needle cast

Quantification of *Dothistroma septosporum* Spores by Real-Time PCR

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Dothistroma septosporum (teleomorph: *Mycosphaerella pini*) is the main causal agent of dothistroma needle blight which is spreading through different areas of the world. Reliable and species-specific methods for quantification of spores are not available for D. *septosporum*. For this reason, a new molecular based method for species-specific quantification of *D. septosporum* spores is being developed using a plasmid as a quantification standard.

This method consists of a real-time polymerase chain reaction (qPCR) during which a partial region of the single copy *D. septosporum* gene, *pksA*, is amplified. This *pksA* region, with 2 variants variable in the length (101 bp and 769 bp), was cloned into a pGEM®-T Easy plasmid for use as a DNA standard. Standard curves were prepared using different dilutions of plasmid copy number (in range of 100 000 – 5 plasmids). Linear plasmid showed lower quantification cycle (Cq) values, in average by about 2 cycles compared to circular plasmid in both length variants of plasmid using a SYBR Green detection system. However, circular plasmid showed the same Cq value and PCR efficiency as linear plasmid using hydrolysis probes. Circular plasmid was chosen for subsequent experiments due to ease of preparation. The lowest detection limit was 5 copies per reaction after adding t-RNA. PCR efficiency was approximately 2.1.

Standard curves of pGEM-*pksA* plasmid will be correlated with standard curves generated from spore solutions of known concentrations. The method will be developed further to allow quantification of spores from environmental samples such as collected on spore traps. It will provide a high throughput method for identification and quantification of *D. septosporum* spores.

Dothistroma septosporum: Incidence of Spore Production and Weather Condition

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Dothistroma septosporum (Dorog.) Morelet. and *Dothistroma pini* Barnes are fungal pathogens responsible for a very serious needle disease Dothistroma needle blight (DNB), also known as Red band needle blight. Woods et al. (2005) considers DNB as a disease supported by global climate change. He emphasizes the influence of locally enhanced precipitations to the spreading of the disease in temperate zone.

Although a number of papers were published about phenology of DNB, no attempt was made to describe the weather conditions, which are necessary for production of conidia and general pattern of conidia releasing during the year. The aim of this paper was to study the temporal spore dispersal patterns of *D. septosporum*.

Spore trapping was done for a period of two seasons from March to December 2009 and 2010 in an 13-year-old (2009) plantation of austrian pine (*Pinus nigra*) in the forest district "Soutok", near Lanžhot, South Moravia, Czech Republic. This plantation has been strongly infected by *D. septosporum* for a few years.

For trapping the Dothistroma spores the automatic volumetric spore trap of usual Hirst, Burkard or Lanzoni VPPS construction was used, described by Hirst (1952), Portnoy et al. (2000) or Konopinska (2004). Our spore trap (AMET Velké Bílovice, Czech Republic) was placed inside the plantation with the orifice 0.3 m above soil level. Close to the spore trap, a SIGNALIZATOR automatic climatic station (AMET Velké Bílovice, Czech Republic) was installed 2 m above the ground.

From the evaluation of sticky tapes from the trap was noticed, that the production of spores occurred solely during days with average day temperature above 10°C and only in the part of season without frost days. Such conditions started in the third decade of April and finished at the beginning of October. During the spore-active season some periods of interruptions were apparent. The longest period without any spore was the first and the second decade of June. It is characterised by average daily temperature above 18°C and average daily relative humidity under 75%. Upon such dry conditions the sporulation ceases within two days. On the other hand, the optimal weather conditions are average daily temperatures 15 – 20°C and average daily relative humidity above 90%. This optimal period was taken from the end of July to the half of September. The highest amount of spores in one cubic meter of air was 3,89. The lowest detectable amount was 0,07 spores/m^3 of air.

The Occurrence of *Dothistroma septosporum* in Different Types of Forests in Finland

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Red band needlecast has found in Finland during the last years. It has been distributed almost allover the Finland, but seems to be most common in the southern and central part of country. The aim of this research is to find out the frequency of red band needle blight distribution in different type of pine stands. Normally the pine stands in dry forest sites are healthy without any needlecast. Although the reasons for disease distribution are unclear, the increased length and increased humidity of growing seasons may favour the dispersal and infection of spores. Earlier epidemics caused by *Lophodermium seditiosum* could outbreak especially in southern Finland, but during the last years red band needle blight has been also caused needlecast in pine stands, too. The amount of red band needlecast varies a lot between sites, depending on the density of trees vegetation and the type of soil etc.

Session 4: Foliage and dieback diseases

Foliage Diseases on True Fir in Norway

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During the last decade, we have observed a number of airborne fungi causing foliage diseases on true fir (*Abies* spp.) in nursery, Christmas tree, bough, and/or landscape plantings in Norway; *Botryotinia fuckeliana*, *Delphinella abietis*, *Herpotrichia parasitica*, *Melampsora abieti-capraearum*, *Phaeocryptopus nudus*, *Pucciniastrum epilobii*, *Rhizosphaera kalkhoffii*, and *Sydowia polyspora*. Fir plantations in Norway are mainly for Christmas trees, a production situated to a large extent in western Norway, where the mild, humid climate is ideal for fungal infection and development.

Botryotinia fuckeliana is mainly a problem in nurseries, but occasionally damaged shoots have been observed in Christmas tree fields due to wet conditions during shoot elongation. We have found damage by *B. fuckeliana* on subalpine fir (*A. lasiocarpa*), nordmann fir (*A. nordmanniana*), Korean fir (*A. koreana*), noble fir (*A. procera*), and white fir (*A. concolor*).

Delphinella abietis destroys current year needles, and in severe cases entire shoots. The needles curl downwards along the edges and are usually covered by numerous, black pseudothecia. We have found the disease on subalpine fir, Turkish fir (*A. bornmuelleriana*), Siberian fir (*A. sibirica*), nordmann fir, and noble fir in western Norway.

Herpotrichia parasitica kills both old and young needles. The stomatal areas of the needles get covered by brown hypha. The needles turn greyish and hang straight down from the twigs, only attached by mycelium. We have seen severe damage in south western Norway on silver fir (*A. alba*) in a forest stand, and on Turkish fir and nordmann fir in Christmas tree fields.

The rust fungi *Pucciniastrum epilobii* and *Melampsora abieti-capraearum* are problematic in Christmas tree fields in years with high precipitation during shoot elongation, given their alternating hosts are present; *Epilobium* spp. and *Salix caprea*, respectively. The former is damaging both nordmann and subalpine fir in southern Norway, while the latter has only been found on nordmann fir.

Phaeocryptopus nudus has been found on *A. lasiocarpa* (corkbark fir and subalpine fir) in southern Norway. It is problematic in the subalpine fir Christmas tree production. The symptoms show up approximately a year after infection, thus, the current year shoots appear healthy, while the older needles turn brown. In severe cases shoots die.

Rhizosphaera kalkhoffii causes needle cast. We have found severe damage on nordmann fir, subalpine fir, and Korean fir in Christmas tree fields in southern Norway. Small, black, globose pycnidia cover the stomatal bands.

Sydowia polyspora is involved in two serious diseases on fir Christmas trees in Norway and elsewhere; "*Sclerophoma* shoot dieback" (conidial stage referred to as *Sclerophoma pithyophila*) and "current season needle necrosis" (CSNN) (conidia stage referred to as *Hormonema dematioides*). The former may kill the entire shoot, while CSNN gives necrotic spots and bands on new needles, often followed by heavy needle cast. We have isolated the fungus from noble fir, nordmann fir, grand fir (*A. grandis*), and subalpine fir.

Does Long Distance Gene Flow Occur Between Subpopulations of *Lophodermium piceae*, the Most Common Needle Endophyte of Norway Spruce?

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Lophodermium piceae is a ubiquitous endophytic inhabitant of Norway spruce needles (*Picea abies*). It can generally be found in the majority of older (> 2 yr) needles of single trees and it may be one of the most numerous fungi in spruce forests. The fungus is transmitted by aerial ascospores, which are formed on dead needles still attached to twigs in the tree crown or on fallen senescent needles. Locally, *L. piceae* is a highly diverse fungus and it is difficult to find identical (characterized by DNA markers) isolates even within a single needle. The aim of this study was to examine the degree of differentiation within and among Eurasian subpopulations separated by various distances and geographical barriers. For this purpose, samples of seven subpopulations (including 14-46 isolates/subpopulation) were collected along a north-south transect stretching from the northern timberline in Finnish Lapland to the southern border of the distribution area of Norway spruce in northern Italy. Additionally, isolates obtained from areas nearby Irkuts, Siberia, were included. The investigation included in total 227 isolates. Differentiation between *L. piceae* subpopulations was determined from DNA sequences of three genetic markers. One of the markers was the internal transcribed spacer (ITS) of the ribosomal DNA and the other two (LP1 and LP2) were sequence characterized amplified regions (SCAR) found in *L. piceae*. Results including sequences of Finnish, Belarusian, Swiss, Italian and Siberian isolates showed low differentiation among populations. According to analysis of molecular variance the among subpopulation variation was 1%, 2% and 3% in ITS, LP1 and LP2 markers, respectively. This low variation among subpopulations indicates high gene flow between them.

White Pine Needle Diseases in Eastern Canada

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In 2009, yellowing of white pine (*Pinus strobus*) needles was reported from several regions in three Canadian provinces: New Brunswick, Québec and Ontario. A similar problem was seen also in eastern United States. Several causal agents were presented as hypotheses: drought, pollution as well as several needle diseases. In 2010, samples of white pine needles were collected in areas where symptoms were seen the previous year. Samplings were done by the three provincial agencies. In addition, one white pine was sampled every month in Québec City and some endophytic fungi were isolated from diseased needles collected in June. At least 6 fungal species were more common on these needles but some were secondary fungi like *Hendersonnia pinicola*. The most common pathogen found was *Canavirgella banfieldii* which seems to be a synonym of *Lophophacidium dooksii.* The yellowing of the current year needles is visible mainly in August. The discoloration affects only a distal portion of the needles. Some white pine seems to be resistant to this disease. The teleomorph is visible mainly on previous year needles in early summer. A second pathogen, *Mycosphaerella dearnessii*, appears in June on previous year needles: the whole needle becomes yellow and red bands are visible near the infection point. Both pathogens were collected on the same tree on few occasions. All these fungi are being sequenced and this should clarify the synonymy at some fungal species level and their classification at the family level.

Cedrus libani, a New Host for *Herpotrichia juniperi*

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Lebanon cedar (*Cedrus libani*) is an ecologically, economically and historically important tree species that has been exposed to severe anthropogenic impacts through intensive cutting, burning and goat grazing over the past 5000 years resulting in an excessive reduction in population size. Today, excluding small and degraded populations in Lebanon and Syria, the primary natural distribution of Lebanon cedar is in the Taurus Mountains of Asia Minor. *C. libani* is one of the most important tree species occurring at high altitudes up to 2100m in Turkey, thereby contributing to the highly diverse protective functions of high mountain forests. Efforts to protect existing forests and natural regeneration of this endangered tree species were undertaken in recent years; however, there is a lack of special techniques to develop and protect high mountainous forest ecosystems in Turkey.
Recently brown felt blight caused by *Herpotrichia juniperi*, was observed for the first time on Lebanon cedar in Turkey. *H. juniperi* is known to have the potential to destroy a whole natural regeneration after a winter with a long lasting, thick snow cover, often combined with snow melt late in spring. This pathogen seems to be an endemic species coevolved with its host species. However, it may play an important role in distribution and existence of *C. libani* and other host species at high altitude forest as a disturbance agent that affects the survival and growth of its hosts. The current situation, possible threats and the impact of climate change on the occurrence and the magnitude of damage of *H. juniperi* is discussed.

Enhancing Systemic Resistance of Maple Against Tar Spot Disease

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Plants are known to possess natural defense mechanisms against stresses including diseases. In cultivated systems or under high disease pressure, these natural mechanisms may be insufficient to guard the plants against disease outbreaks. There are chemicals that have been observed to stimulate the natural resistance pathways in plants. We have been investigating the mechanism of action of certain new compounds in their role of defense activation against diseases in plants, including tar spot of maple caused by *Rhytisma* species. These compounds generally do not have strong direct anti-fungal effects, but activate signaling pathways within the plant to either cause direct expression of defense-related genes prior to pathogen attack (induction) or allow expression of defense-related genes more quickly in response to pathogen attack (priming). Using tests in the lab and in the field, we have found that applications of such chemicals either alone or in combination can reduce plant diseases significantly. This presentation will explore the use of such chemicals for plant disease control, and discuss their advantages and possible disadvantages.

Fungi in Shoots and Foliage of *Fraxinus excelsior* and *F. angustifolia* in Eastern Ukraine

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During the last years, the massive dieback of ash (*Fraxinus* spp.) caused by *Hymenosyphus pseudoalbidus* is observed over large areas of Europe. Until 2010, there was little concern regarding ash health condition in Ukraine, as no dieback symptoms were reported. During the last year, however, some morphological symptoms of ash decline have been observed in the eastern part of the country: uneven flushing, occasional shoot necroses, discoloration of wood and premature leaf-shedding. The main aim of our work was to investigate fungal communities in necrotic and healthy-looking shoots, and in petioles of leaves that were shed during previous vegetation season. The detection of fungi has been accomplished using molecular methods: direct extraction of DNA from plant tissue, its amplification using PCR with ITS primers, subsequent sequencing, and comparison of the sequences with the sequences of fungi originating from disease-devastated areas.
The 176 samples symptomatic and healthy (i.e. having necrotic lesions) of shoots and leaves were collected for molecular identification of fungal community and isolation of fungal cultures.
In addition, health condition of different ash provenances was visually assessed on seven monitoring plots (24 test trees in every plot) at two locations in eastern Ukraine. The plots represented ash stands different ages (from 10 to 80) with different crown condition. The results will be reported.

Ash Dieback in a Seed Plantation in Austria

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Dieback of common ash (*Fraxinus excelsior*) is caused by the recently described ascomycete fungus *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*). The high intensity and the gradual appearance of the disease in Europe, related to the successive spread of the causative pathogen, may suggest that *H. pseudoalbidus* is an alien invasive organism. Observations of healthy and only slightly affected trees in severely diseased ash stands may reflect considerable differences in resistance between individuals of *F. excelsior* to the ash dieback pathogen.

In 2009 and 2010 the intensity of ash dieback was investigated in an ash seed plantation located in Feldkirchen an der Donau (14.02 E, 48.20 N, 264 m asl., province Upper Austria, Austria). In this 1.36-hectare-large plantation, initiated in 1993, 51 different ash clones of local provenance (forest ecoregion 7.1, northern foothills of the Alps, western part, submontane altitudinal zone) are represented with two to four grafted ramets each. For disease assessments, the crown of each individual tree was divided into three equal parts. Each crown third was assigned to one out of six ash dieback severity classes (0, no dieback of shoots, twigs and branches; 1, < 5 % dieback; 2, > 5 % to 20 % dieback; 3, > 20 to 50 % dieback; 4, > 50 to 80 % dieback; 5, > 80 % to < 100 % dieback; 6, 100 % dieback). Using class means (0, 2.5, 12.5, 35, 65, 90, 100), the values of the three crown thirds were averaged, to obtain an ash dieback severity rating (in percent) for each ramet. Leaf shedding, another symptom of ash dieback was rated in a similar way as dieback of shoots, twigs and branches. In both years assessments were done in mid-May, at the end of July and in early to mid-September.

Considering all 187 evaluated trees, mean ash dieback intensity was 18.1 % in July 2009 and 17.6 % in July 2010. Disease intensity varied considerably between clones, ranging in both years from almost no dieback to more than 80 % dieback in the most severely affected clone. In May 2010 mean dieback intensity was below 10 % in 28 out of the 51 clones (55 %), between 10.1 and 20 % in 13 clones (25 %), between 20.1 and 30 % in 5 clones (12 %) and between 30.1 and 40 % in one clone (2 %). Only 4 clones (8 %) had average values higher than 40 %. The assessments clearly suggested that early leaf shedding is a symptom of ash dieback. In both years, the mean percentage of shed leaves was low in July (8 % in 2009, 1 % in 2010), but high in late summer (67 % in early-September 2009, 46 % in mid-September 2010). As it was observed for dieback, intensity of leaf shedding varied considerably between clones, but no obvious relationship between leaf shedding and dieback intensity was detected.

In conclusion, the results may indicate that *F. excelsior* clones indeed show different resistance levels to *H. pseudoalbidus*. However, a longer observation period is needed to monitor disease development in this seed plantation in Austria. Moreover, artificial inoculation of *H. pseudoalbidus* onto leaves and shoots of various clones showing contrasting damage levels are desirable in order to confirm that resistance against this emerging tree pathogen varies in *F. excelsior*.

Session 5: Bark diseases

Impact of Beech Bark Disease on the Sustainability of American Beech in New York

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Beech bark disease (BBD) is a disease complex involving the beech scale (*Cryptococcus fagisuga*) and canker fungi in the genus *Neonectria*. Beech bark disease has affected American beech (*Fagus grandifolia)* in eastern North America since its introduction in the 1890s. Northeastern forests are comprised of diverse tree species. Factors associated with tree species composition could affect the distribution of the BBD causal agents, which in turn could impact the sustainability of beech. A sustainable forest ecosystem was defined as one that offsets current growth with current mortality, thus maintaining a stable size-structure relationship. Data from 539 plots with beech, containing 2,495 beech trees, were analyzed. Eleven forest types with beech were identified. For American beech populations within each forest type, the baseline mortality required to maintain a stable size-structure relationship was compared to the observed mortality. Consistently greater than predicted mortality in the mid to large diameter (dbh=31-46 cm) classes indicate sustainability problems in these dbh classes for beech populations in the sugar maple (*Acer saccharum*)-beech, red maple (*Acer rubrum*)-beech, and eastern hemlock (*Tsuga canadensis*) forest types. Cutting has contributed to the mortality in dbh classes >26 cm in the sugar maple-beech forest type, which has the greatest proportion of beech (43% of all trees). Beech regeneration is abundant and BBD-free trees were present even in the large DBH classes, suggesting the future build up of a resistant population.

Neonectria sp., a New Pathogen Causing Cankers on Norway Spruce?

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From 15 to 30 year-old Norway spruce stands have been found to suffer from cankers and necrotic lesions on stems in different parts of Finland. Fungal isolations from the margin of healthy and diseased tissue resulted in many fungal species, but the only common species was *Neonectria* sp. In inoculations done with agar blocks taken from two-week-old cultures on MEA, *Neonectria* sp. was shown to be pathogenic to one- and two-year-old Norway spruce seedlings.

Neonectria-Canker on Trees in Norway

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Neonectria spp. have been found on a number of tree genera in Norway; *Abies*, *Acer, Cornus, Fraxinus, Ilex, Malus, Picea, Populus, Prunus, Pyrus, Sorbus*, and others.
Neonectria canker is a serious problem in Norwegian apple orchards, and in 2010 we investigate whether host plants other than apple trees were potential inoculum sources. We sequenced the ITS-regions from *Neonectria* cultures isolated from apple trees and two other host plants in the rose family (*Rosaceae*); *Sorbus aucuparia* and *Prunus padus*. Cultures from all 3 hosts produced floccose, white mycelium. The cultures had identical sequences, and they were identical to sequences of *N. ditissima* (syn. *N. galligena*) deposited in GenBank. Inoculation tests have not yet taken place, thus, we have no indications that cross infections take place in nature. Further research is needed to find out if infected *S. aucuparia* or *P. padus* in the vicinity of apple orchards may increase the disease pressure.
Interestingly, cultures isolated from conifers in Norway in 2008, differed from *N. ditissima* by five out of the 550 base pairs included in the ITS-sequence. Previously *N. fuckeliana* has been reported on spruce species in Norway, and on spruce and fir species in other countries, but *N. fuckeliana* differs by more than 20 base pairs from the *N. ditissima*-like isolates we obtained from conifers in 2008. The *N. ditissima*-like fungus might be a new species related to *N. ditissima*, possibly imported to Norway.
N. ditissima has to our knowledge never been described as a pathogen on conifers, but the *N. ditissima*-like fungus we isolated from conifers in 2008 was pathogenic. We first discovered a serious disease outbreak on white fir (*Abies concolor*) in southern Norway, and the *N. ditissima*-like fungus was isolated from dying trees in two counties in south western and four counties in south eastern Norway. Both old and young trees were dead or dying. The *N. ditissima*-like fungus was later isolated from Siberian fir (*A. sibirica*), subalpine fir (*A. lasiocarpa*), and Norway spruce (*Picea abies*) in south eastern Norway. Sequencing showed that all the *Neonectria*-isolates from different conifer hosts in 2008 were identical in their ITS-region. Perithecia in canker wounds from the conifer samples were dark around the ostiole. This morphological characteristic is known from *N. ditissima,* but not from *N. fuckeliana*. Cultures from *N. fuckeliana* are brownish.
Inoculation tests with *N. ditissima*-like isolates were carried out in 2009 on subalpine fir, white fir, and Norway spruce, and the fungus was pathogenic on all three species.

Pathogenicity of some Fungi Isolated from Cankers on *Cupressus sempervirens* var. *horizontalis* in Turkey

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Natural stands of *C. sempervirens* in Turkey are among the largest forests of this species in the world and are regarded as relicts of the centre of origin of var. *horizontalis*. In this study we tested the pathogenicity of some of the most common fungal isolates originating from cankers on *C. sempervirens* by inoculating the isolates into the inner bark of *C. sempervirens* saplings. The internal transcribed spacer (ITS) region of rDNA of the isolates was sequenced and compared with those in GenBank. Among the isolates eight ITS taxa were found. The isolates were inoculated into the inner bark of 2-year-old *C. sempervirens* seedlings, on average 90 cm tall and 6-12 mm thick at the base. The seedlings were incubated seven weeks in a growth chamber at 70% mean relative humidity and 22.5 ºC mean temperature. The coaxial length of the lesion around the inoculation point on each seedling was measured. Among the eight ITS taxa *Pestalotiopsis funerea*, two other species of *Pestalotiopsis*, and two unidentified species belonging to the class Dothideomycetes caused lesions that were significantly larger than those in the controls while *Fusarium* sp., *Cytospora* sp. and an unidentified species belonging to mitosporic Amphisphaeriaceae did not. In contrast to the Pestalotiopsis species, the two members of Dothideomycetes grew also into the sapwood of the seedlings.

New Alternate Hosts for *Cronartium* spp. in Finland

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Cronartium flaccidum causes serious rust epidemics on *Pinus sylvestris* in northern Fennoscandia. The rust spreads via *Melampyrum* spp. in diseased stands. The main alternate host for the rust in genus *Melampyrum* is *M. sylvaticum* in northern Finland. The rust occurs also commonly on *Vincetoxicum hirundinaria* in southern Finland, but it has also been found on *Pedicularis* spp. and *Paeonia* spp. in natural forests and garden plants in Finland. In artificial inoculations, the rust has been shown to infect several other species in other genera elsewhere in Europe and Asia.
Cronartium ribicola is most common on five-needle pines in arboretums and botanical gardens in southern Finland. The rust spreads via a high number of cultivars of *Ribes* spp. The rust is known to be very host-specific in Europe.
A number of inoculation experiments were conducted in the laboratory and greenhouse to test the susceptibility of alternate hosts in potential plant genera to *C. flaccidum* and *C. ribicola* in 2008-2010. Both *C. flaccidum* and *C. ribicola* formed uredinia and telia on *Pedicularis palustris* ssp. *palustris*. Either uredinia or telia of *C. flaccidum* and *C. ribicola* developed also on several earlier unreported species in previously unreported families.
The high number of new alternate hosts capable of spreading the rust shows the low host specificity of *C. flaccidum*. The results suggest that the virulence of the European *C. ribicola* is much wider than earlier reported. Sampling of natural samples of the new alternate hosts is needed to clarify their role in spreading rust epidemics in practice.

Differences in twig endophyte assemblages between native black poplar (*Populus nigra*) and a cultivated hybrid poplar (*Populus x euramericana*)

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The European black poplar (*Populus nigra*) is considered one of the most seriously endangered indigenous tree species in Europe. The question remains whether a possible extinction of *P. nigra* through replacement by *P x euramericana* or a possible drastic decrease in genetic variation through intensive introgression would imply the extinction of those organisms that are obligatory associates of *P. nigra*. To achieve this, we compared the endophytic mycota in twigs of native poplar (*P. nigra*) and hybrid poplar plantations (*P. x euramericana* clone I-214). Twig endophytes were isolated from native and hybrid poplar stands in Palencia (N. Spain), and identified according to sequences of the internal transcribed spacer (ITS) region of their rDNA. The results revealed that the endophyte community on poplar forests may be affected by an extinction of *P. nigra* or a drastic decrease in genetic variation, because it differed considerably between native and hybrid poplars of this study.

Session 6: Other diseases I

The Potential of Soil Bacteria and their Biosurfactants to Suppress *Phytophthora* Diseases of Forest Trees

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Diseases caused by the fungi-like organisms, *Phytophthora* spp. (Oomycetes), are a major problem in deciduous forests. Alarmingly, problems with *Phytophthor*a-diseases are expected to be further magnified and intensified in the future, since the conditions under the predicted climate change are likely to favor the growth and spread of these pathogens also in the Northern Europe. New control strategies are thus urgently needed. The use of microorganisms as biocontrol agents is an environmentally sound alternative to chemical pesticides and could be an integrated part of sustainable management of *Phytophthora* pathogens of forest trees. A group of microorganisms with high potential in biocontrol are the fluorescent pseudomonads (*Pseudomonas* spp.). They are indigenous in the environment and may excrete metabolites that are inhibitory to pathogenic microbes. An important group of metabolites produced by fluorescent pseudomonads are biosurfactants. Due to their zoosporicidal activity, these chemicals have attracted increased attention as a potential tool in biocontrol of pathogens belonging to Oomycetes. We have initiated studies to evaluate the potential of biosurfactant-producing pseudomonads to protect deciduous forest trees against *Phytophthora* spp. First, we used in vitro tests to validate that biosurfactants produced by a fluorescent pseudomonad, *P. koreensis* 2.74, isolated from a horticultural system, would effectively lyse the zoospores of oak pathogenic *P. quericina*. Second, we conducted a greenhouse experiment with young oak seedlings to study whether the mortality of oak seedlings due to P. quercina infections could be reduced by treating the plants with biosurfactant-producing pseudomonads. Third, we investigated whether the treatment with bacteria could also induce alterations in the potentially defensive phenolic metabolites in oaks. The implications of the results for control strategies in nurseries and natural habitats are discussed.

Phytophthora Diseases of Chestnut Trees in Black Sea Region of Turkey

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Phytophthora disease of chestnut trees in Black Sea region of Turkey was investigated by using 85 soil and root samples collected from the trees showing dieback symptoms. Samples were mainly collected from chestnut trees, showing more intensive dieback symptoms. In addition, presence of *Phytophthora* diseases was investigated in important forest nurseries growing chestnut trees. *Phytophthora* spp. in the chestnut forests and nurseries was determined by using baiting technique with the younger leaves of the chestnut saplings and direct plating of diseased roots on selective media respectively. *Phytophthora* spp. were identified by using morphological and cultural aspects of the isolates and by analysing their gene sequences of their ITS regions. In the chestnut forests of this region the following *Phytophthora* spp. were recovered; *Phytophthora cambivora*, *P. cinnamomi, P. citricola* and *P. crytopgea*. *P. cinnamomi* was also found in 3 nurseries. The most widespread species on chestnut was *P. cinnamomi*. All the *Phytophthora* species were found pathogenic on its host. The most aggressive species on chestnut were *P. cambivora*, *P. citrophthora* and *P. cinnamomi*.

Susceptibility Assessment of Common Alder Seedlings to *Phytophthora alni* and Other *Phytophthora* Species

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Common alder (*Alnus glutinosa*) (L.) Gaertn seedlings were tested in vitro for their susceptibility towards alder pathogen *Phytophthora alni* and other *Phytophthora* species. Isolates of *P. alni* and other *Phytophthora* species (*P. cinnamomi*, *P. citrophthora*, *P. nicotianae* and *P. palmivora*) were used in the assay. Seedlings were inoculated with uniform mycelial blocks of agar. Susceptibility was assessed in terms of seedling mortality (%) after 67 days of inoculation. Seedlings were found highly susceptible to *P. alni* and also to other *Phytophthora* spp. varied from higher to lesser extent. Results implied that common alder seedlings are at risk to be infected by *Phytophthora* spp. and showed relative host-nonspecificity of this genus.

Research on Oak Decline Disease in Spain

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Holm oak and cork oak are the two *Quercus* species affected by oak decline (named "seca" in Spanish) in Spain. *Phytophthora cinnamomi* is the soil fungus associated with this disease. Since 1991 this fungus has been isolated from feeder roots of both oak species and after analyzing more than 700 soil and root samples, all from oak areas with a considerable number of damaged trees, it is considered the main cause of oak decline. Disease symptoms have been obtained by inoculating mycelium and zoospores of *P. cinnamomi* both in adult trees in the field and in seedlings (two years old) in the greenhouse. Studies on disease dynamics (zoospore production, influence of cations and anions in fungal development, influence of soil moisture, host-pathogen interaction, and zoospore infection) have been carried out over the last fifteen years both in the laboratory and the greenhouse. In addition an experimental plot containing the Spanish *Quercus* species has been planted in a high soil contaminated area by *Phythopthora*, and monitored during the last years. A summary of all this study lines will be presented, and the results of sampling campaigns done in the affected areas during the last 20 years.

On the Use of *Chondrostereum purpureum* in Controlling Hardwood Sprouting

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Hardwood sprouting is a considerable problem in forest regeneration, road sides and also under electric lines. As chemical control in not used any more due to environmental problems and public opinion, biocontrol has become an interesting option.
There is a biocontrol product available in Canada, but importing North American pathogen to Europe may have risks, and probably would not even satisfy the public opinion. Therefore we have studied possibilities to use Finnish *Chondrostereum purpureum* isolates for this purpose.
Our results on birch (*Betula pendula* and *B. pubescens*) have shown, that sprout control efficiencies of more than 80% may be reached using natural strains. Already this figure might be satisfactory for practical application, but we intended to test, if it could be increased by breeding. This project is still under its way, but preliminary first generation results do not show considerable improvements.
In Nordic countries also *Phlebia gigantea* is used for biocontrol in forestry. In this project, we tested, whether both biocontrol agents (*C. purpureum* and *P. gigantea*) could be used with the same equipment, or if small residuals of one agents hampers the other. The results showed that the viability of both fungi decreased when small amounts of the other one was added. However, in efficacy testing the control of both fungi surprisingly increased against both hardwood sprouting or *Heterobasidion parviporum*, respectively.

The Soil Temperatures During Prescribed Burning and the Occurrence of *Rhizina undulata* Fr.

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Rhizina undulata is a postfire fungus. The ascospores germinate after heating over 35 °C. The prescribed burning of one forest compartment was done in the purpose to investigate the effects of forest fire to atmosphere at Hyytiälä Forestry Field Station in Southern Finland. The soil temperature measurements were one part of that research. One year after burning the ascocarps of *Rhizina undulata* appeared offering the possibility to use temperature data for studying the ecology of the species. The soil temperature was measured with 21 iButton sensors. Before and during the burning all sensors were at 7 cm depth in the burned area. After burning 10 sensors were moved to the unburned control area. The ascocarps were inventoried from 40 systematically located 10 m^2 plots. In average 6 ascocarps were found in one plot. Ascocarps were present on 75 % of plots. Most ascocarps were found on spots for seeding with bare mineral soil visible. The fungus has not killed germlings of pine but 43 % of planted seedlings were dead. The temperature during the burning reached 35 °C in 10 points. In 4 points the temperature was over 60 °C, which could be too high temperature for spores to survive. It seems that the ascospores should exist in the soil in 2 – 10 cm depth if they can germinate. The temperatures after burning did not reach 35 °C in 7 cm depth. More measurements are needed to show if the temperature in the surface of burned area reached critical point after burning.

Using Genomics to Gain Insights into the Evolution and Biology of *Pseudomonas syringae* pv. *aesculi* on European Horse Chestnut

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Pseudomonas syringae pv. *aesculi* (*Pae*) causes a devastating bleeding canker disease of European horse chestnut (*Aesculus hippocastanum*) across several countries in northwest Europe. The pathogen can enter woody branches directly *via* lenticels, leaf scars and other discontinuities in the bark, causing lesions in the cortex and phloem. Cankers can expand rapidly, causing bleeding symptoms on the stem and branches, and the trees suffer progressive crown dieback often leading to mortality. Current studies also suggest that *Pae* can survive for long periods in soil and remain viable after freezing. To gain insights into the evolutionary and biological adaptations of *Pae*, the draft genome sequences were generated for four strains of *Pae* including three strains from Britain and a type strain from India that causes leaf spot on Indian horse chestnut (*Aesculus indica*). The genomic data suggest that the British and Indian *Pae* strains share a very recent common ancestor and that the three British *Pae* strains descend from a single, very recent introduction of the bacterium into Britain. Southern blotting of insertion sequence elements for a broad range of *Pae* strains is currently providing further information on the pathogen's geographical pathways of spread within Europe. A phylogenetic analysis based on a set of conserved genes showed that *Pae* belongs to a distinct clade of *P. syringae* pathovars adapted to woody hosts. Genomic comparisons with other *P. syringae* pathovars showed that *Pae* has acquired genes that may enable it to infect and live within the woody parts of the tree. These include genes which code for enzymes involved in the degradation of plant-derived aromatic compounds, and others which likely have a role in disabling the tree's defence responses. These genes have not yet been found in other pathovars of *P. syringae* that infect herbaceous plants but may be conserved in other tree-infecting bacteria and thus, may be important to our understanding of the infection processes of bacterial tree diseases.

Sequencing and Assembly of a Fungal Genome

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With the Next Generation Sequencing technologies, it is now feasible, within a small research program, to attempt de novo sequencing and assembly of small eukaryotic genomes (<100 Mb in size). In late 2009, we decided to attempt sequencing of the genome of the fungus *Colletotrichum cereale*. Since this species had been split from *C. graminicola* in 2006, and the rough genome assembly of *C. graminicola* became available in 2008, we thought that these two genomes would be similar enough for *C. graminicola* to act as the reference genome for sequencing of *C. cereale*. We prepared genomic DNA of an isolate of *C. cereale* using standard methods, and sent in 10 ug as requested. The sequencing center then took two weeks for library construction, and another two weeks to run out on a Illumina GAIIx sequencer. They generated ~25-fold coverage in 35 bp paired-end reads of this 60 Mb genome (1.5 Gb worth of sequence data plus 4 Tb worth of image files and other data associated with sequencing). They attempted assembly of the 35 bp reads, based first on the *C. graminicola* genome and then other fungal genomes, and then provided the results. I then also attempted assembly of this data with a variety of programs and a multitude of settings. With genomes of species that have already been sequenced, the new sequencing technologies may be feasible to obtain the genome of an isolate of the same species; but for species lacking a reference genome, the technology in terms of software and perhaps equipment may have limitations. The sequencing technology and the results of this attempt at fungal genome sequencing and assembly are discussed.

Phytosanitary Conditions of *Quercus cerris* in Tuscany Evaluated by Monitoring, GIS Applications and Molecular Techniques

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Data from the phytosanitary monitoring program in Tuscany (META) collected during the last four years showed an increasing of number *Quercus cerris* stands affected by *Biscogniauxia mediterranea* (from 12% to 78%) followed by a two-years period of decreasing values.
Since the occurrence of this fungus is considered as a good indicator of Oak stress in central Italy, a study was performed to verify whether environmental parameters are involved in oak decline.
The study was based on the use of GIS technology and was validated as fungal presence from symptomless oak twig samples. Fungal detection was assessed by using isolation and real-time PCR, already optimized from this research group in a *B. mediterranea*/*Quercus* pathosystem.
During summer 2009 geographical and monitoring data were obtained from twenty Oak areas in Tuscany. Stands dendrological information and crown conditions evaluated according to ICP-Forest monitoring method were registered; damages and severity index for crown damages were also calculated. Finally, all data were stored and managed by a GIS system by ArcMap 9.3 software.
Using climatic data, obtained from official local meteorological networks, the mean temperatures and total rainfall during the vegetative seasons were calculated and then data points were interpolated by using Ordinary Kriging technique to obtain prediction climatic maps at local scale. Vector maps of chemical, physical and biological properties of soil according to USDA classification, digital elevation model, viability map and *Q. cerris* regional distribution map were lastly stored into GIS system. Results of monitoring showed that the most common damages observed were diebacks and necrosis of branches. Kruskal-Wallis test allowed to grade the areas on the base of severity of crown damages level.
Results of laboratory analysis confirmed that samples collected from damaged areas hosted a significantly higher amount of *B. mediterranea* DNA in oak asymptomatic tissue, compared to areas with lower damages. All geographical and climatic data stored in GIS system used as predictors. Temperature and soil characteristics showed inverse relationship with crown damages in multiple regression model, while rainfall, elevation, and quantitative of clay and sand were positively linked to crown damages.

Evaluating Methyl Jasmonate for Induction of Resistance Against *Fusarium oxysporum*, *F. circinatum* and *Ophiostoma novo-ulmi*

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Worldwide, damping off is probably the most severe disease affecting seedlings in forest nurseries. In Spain, the pitch canker and the Dutch elm disease are amongst the diseases causing higher economic looses in forests, mostly in adult trees. Exogenous applications of Methyl Jasmonate (MJ) have been successfully used to artificially induce chemical defense responses and resistance of forest trees against several insects and pathogens. MJ is usually mixed with the surfactant Tween 20 (0.1% v/v) and applied by spraying or by brushing the plant stem. Conscious that induced resistance occurs in pines and elms, we conducted three different experiments to evaluate if MJ induces resistance in *Pinus pinaster* against *Fusarium oxysporum* and *F. circinatum*, and in *Ulmus minor* against *Ophiostoma novo-ulmi*.

First, different concentrations of an aqueous solution of MJ were applied to *P. pinaster* seeds and seedlings. Seeds were treated by (i) spray with 5mM of MJ solution, (ii) immersion 10-minutes in 5mM of MJ solution, or (iii) spray with water. During sowing, seeds were inoculated with *F. oxysporum* (5 mL; 10^5 and 10^7 spores mL^{-1}) onto the ground. In non-inoculated seeds, MJ treatments did not significantly affect seed germination but originated 44% of seedling mortality 1 month after treatments. In inoculated seeds, MJ treatments caused a significantly higher mortality by *F. oxysporum* in relation to the control treatment (P=0.008). Seedlings were treated with MJ solutions (0, 0.1, 0.5, 1, 5 and 10 mM) by spraying or brushing the stems. Time-periods between treatments and challenge inoculations (10^5 and 10^7 sp/ml) were 1 or 7 days. Seedlings treated at doses above 1 mM MJ showed symptoms of toxicity. Seedlings treated at doses below 1 mM showed higher mortality rates than untreated seedlings, regardless the time of treatment, dose of treatment, inoculum concentration or time of inoculation.

In the second experiment, 0.5-year-old *P. pinaster* seedlings were sprayed with 0, 10 and 25 mM of MJ. One month after treatments, half of the seedlings were inoculated by contact mycelium of *F. circinatum* with a wound onto the stem. MJ did not increase resistance of trees against *F. circinatum*, and no changes in the xylem vessel size or in the xylem vessel density of the treated trees were observed if compared to the controls.

Finally, 5-year-old *U. minor* trees were sprayed with 0, 50 and 100 mM of MJ. Half month after treatments, half of trees were inoculated with *O. novo-ulmi*. 100 mM of MJ was slightly toxic to the trees, causing leave spots and some wilting. However, time to bud burst and tree growth was not altered by MJ treatments. Dieback symptoms, evaluated 120 days and one year after inoculations, revealed that MJ did not protect the plants against *O. novo-ulmi*. In fact, one year after inoculation the trees treated with MJ showed higher dieback symptoms than the control trees (P=0.03).

MJ did not induce resistance in *P. pinaster* seedlings against the two pathogens, probably because plants were too young for the physiological mechanisms responsible for resistance to be operative. Thus, the use of MJ to prevent damping-off in forest nurseries or as a treatment to prevent pitch canker in young seedlings should be discarded. Both *F. circinatum* and *O. novo-ulmi* are well known as highly virulent pathogens of trees. Results suggest that the spread of these pathogens within the trees is faster than the formation of effective defence responses induced by MJ application. However, based on the lack of anatomical and phenological changes observed in our treated plants, in contrast to previous literature, there is no evidence that MJ would cause induction of resistance against the pathogens studied.

Session 6: Other diseases II

Semiochemicals for Monitoring and Control of the Pine Wood Nematode Vector *Monochamus galloprovincialis* (Coleoptera: Cerambycidae)

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Monitoring and control of *Monochamus galloprovincialis*, the sole known cerambycid vector of the pine wood nematode *Bursaphelechus xylophilus* in Europe, are among the most promising strategies against this disease. Recent research has unravelled the very complex, chemically mediated, reproductive behaviour in *M. galloprovincialis.* Males and females locate suitable hosts for feeding and oviposition by responding to host and bark beetle semiochemicals. Then, a male emitted pheromone increases the chance for both sexes to meet on the host tree and contact chemical recognition of females is performed before mating. Such findings led to the development of a very efficient attractant lure, consisting in a blend of the aggregation pheromone plus the kairomones, which is already available to researchers and managers. There exist many potentialities of this tool in pine wilt disease integrated management programs along Europe. Determination of dispersal range and mass trapping of the sawyer are among the most relevant. Two dispersal experiments based on the recapture of marked-released adult insects were carried out in natural and reforested pine stands. Recapture in the pheromone-kairomone baited traps was highly successful, accounting for 33.9% and 29,5% of the 174 and 350 released beetles respectively. Both experiments showed that most insects have a low/moderate dispersal, as some 75% of the beetles was recaptured less than 100m or 141m away, but 6.7% of them were caught at 500 and 6.86% more than 760 m away. One beetle was caught 1.5 km from the release point in the first experiment. Time elapsed between the release and recapture was extended to 91 and 84 days respectively, showing a high life span for *M. galloprovincialis* adults. Results on one mass trapping experiment to test the rate of population extraction and on an operational mass trapping program aimed to vector eradication in the 2008 focus at Sierra de Dios Padre (Extremadura, Spain) are also commented.

Changes of the Structure in a Turkey-Oak Forest after a Tree Decline in North-Hungary

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Similarly to other European countries there was an oak decline at the end of 70's in Hungary; 68% of the *Quercus petraea* and 16% of the *Q. cerris* specimens of the forest's trees died at the Síkfőkút Project, which is covered by a Turkey-oak forest (*Quercetum petraeae-cerris*). This decline resulted in an opening in the canopy; the canopy cover decreased from 80% (1972) to 36% (2007). The aim of our study was to analyse the structural changes in the forest interior after the oak decline. We were focusing on those specimens which were higher than 1.0 m (so-called high shrubs). Height and diameter of each shoot of the high shrubs were registered in a 48×48 m sample area; their location and foliage cover were mapped from 1972 every 4-5 years. Our hypotheses were the followings. (i) After the oak decline in the canopy gaps a secondary tree layer was formed by certain tree species which were grown out from the shrub layer. (ii) Despite of the high number of died oak specimens, the original forest structure did not change considerably. In this process the secondary tree layer played an important role. Only one new tree species, *T. cordata* lived permanently in the site with one specimen from 1988. We found that after the oak decline a secondary tree layer was formed by three new tree species (*A. campestre*, *Cornus mas* and *A. tataricum*). In 2007 there were 130 specimens of *A. campestre*, 22 specimens of *C. mas* and 4 specimens of *A. tataricum* between 8-13 m height and created a secondary tree layer below the primary tree layer of oaks. The average cover of *A. campestre*, *C. mas* and *A. tataricum* increased after the tree decline. The ratio of these species density was only 5.6% of all high shrubs in the secondary tree layer. The canopy cover was 32.4% of high shrubs cover in this new layer. We found negative correlation between the number of died oak specimens and three new tree species density in the secondary tree layer. Negative correlation was between oak tree density and average sizes of new trees. Our study revealed that the forest responded to the tree decline by structural changes in the shrub layer and three new tree species of secondary tree layer compensated the remarkably loss of tree canopy cover.

How to Recover the Health of Secondary Forest "Satoyama" Declining by the Japanese Oak Wilt

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The Japanese oak wilt caused by *Raffaelea quercivora* is increasing year by year in Japan in the secondary forests surrounding rural communities, "Satoyama", from 1990s. Based on the factors promoting the recent oak decline, the method to recover the forest health was discussed. This wilt disease is occurring in aged "Satoyama" forests that had been used for fuelwood and charcoal production and then left unmanaged from the energy revolution started in 1950s. Most oak stands are in the range of 40- to 70-year-old because the traditional coppicing by periodical cutting of 15 to 30 years intervals had stopped by 1980. An ambrosia beetle, *Platypus quercivorus* which vectors *R. quercivora* from dead to living oak trees, propagates effectively in the trunks thicker than 10cm. Due to the extensive population growth of this beetle in aged "Satoyama" forests, infested area is enlarging every year. The eradication of this disease with pesticides is almost impossible in the severely damaged area.

There is a view that damaged stands can be left untouched because those stands will recover naturally for their resilience. However, drastic change is occurring in the vegetation. Itô et al. (2008) clarified shrubs and small-tall trees, or short-lived species are dominantly replacing in the damaged stands. Deterioration of biodiversity and soil erosion are also concerned. To reduce oak mortality in "Satoyama" forests, rejuvenation of trees will be effective because vector beetle cannot propagate in the thin oak trunks. In the stands where wilt infection has not started, clear cutting (narrow area ca. 0.1ha) promotes regeneration of oak trees by the sprouting from stumps. We are recommending this method to local governments and NPOs that are trying to re-manage once abandoned Satoyama. The activities of Satoyama re-management, unfortunately, are sometimes promoting decline for the lack of knowledge on forest health. For instance, thinning of a stand leaving thick oak trees attracts *P. quercivorus*. Cut oak logs abandoned in the stands are helping propagation of *P. quercivorus.* To recover healthy "Satoyama", forest management combined with the utilization of biomass and the contribution of the people of the district will be important. The authors are conducting a social experiment to recover healthy "Satoyama" in the Kansai district. In the experiment, people of the district manage Satoyama forests by themselves cooperating with researchers who provide them with techniques suitable to recover healthy forests. In addition, the effects of biomass utilization as fuelwood in the districts are monitored.

Climate Change and Forest Diseases: Using Today's Knowledge to Address Future Challenges

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The health of the earth's diminishing forested land base is increasingly challenged by the outcomes of human activities, including global climate change. As climate changes, the role and impact of forest diseases on forest ecosystems will also change. Knowledge of relationships between climate variables and forest diseases will be reviewed, with specific emphasis on those affecting foliage, shoots, and stems. Outbreaks of forest diseases caused by native and alien forest pathogens are predicted to become more frequent and intense – this and other general predictions about the effects of climate change on forest diseases will be discussed. Evidence that forest diseases are already responding to the earth's changing climate will be examined (e.g., Dothistroma needle blight in northern British Columbia) as will predicted future scenarios for changes in impact on forests by other tree diseases. Despite the uncertainty that accompanies such predictions it is imperative that researchers, forest managers, and policy makers work together to develop and implement management strategies to enhance the resilience of the worlds' forests. Strategies to be discussed include monitoring, forecasting, planning, and mitigation.

Poster presentations

Effects of Leaf Spotting Caused by Mycosphaerella Leaf Disease and Eucalyptus Rust on *Eucalyptus globulus* in Uruguay

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Mycosphaerella Leaf Disease (*Mycosphaerella* spp. and *Teratosphaeria* spp.) and Eucalyptus rust (*Puccinia psidii*) are important diseases of eucalypt plantations in Uruguay. *Eucalyptus globulus* is highly susceptibility to both diseases; however production losses caused by them have not been properly quantified in this country. In this study, the severity of foliar damage caused by Mycosphaerella Leaf Disease and Eucalyptus rust and the long term effects on growth and survival were assessed in a progeny test of *E. globulus* located in Rocha, Uruguay. The severity of leaf spots was quantified eight months after planting and tree growth and mortality were evaluated two, four and six years later. The trial presented a high incidence of spotting (88.2% of trees showed leaf spots), with a mean severity of 28.7%. The greatest impact of foliar damage, both on growth rate and mortality, occurred in the first two years after damage was assessed. During this period, spot severity less than 40% did not affect growth rate, while survival was affected by spot severity of 70% or higher. When spot severity reached 80% or more, a loss of up to 25% in diameter and an accumulated mortality of 71.7% were registered by the sixth year. It is concluded that, under the intensive Uruguayan productive conditions, *E. globulus* trees tolerate a relatively high degree of leaf spotting. However, severe foliar damage in the first months can cause considerable production losses, compromising the success of the plantation.

Can Global Warming Affect the Survival and Impact of *P. alni* subsp. *alni*?

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The impact of a pathogen on its host is given, among others, by amount of surviving inoculum. The successful survival of *Phytophtora alni* subsp. *alni* which causes devastating epidemics in black and grey alders – the main component of riparian stands – can be affected by winter temperature for several reasons: (1) absence of chlamydospores as potential resting structures, (2) failing of oospore germination connected with meiotic irregularities in this hybrid, (3) reported poor survival ability in soil and rhizosphere, (4) supposed sensitivity to low temperature based on field observations, ecological modelling and laboratory experiments carried out recently. The aim of the work was (1) to study the survival rate of *P. alni* subsp. *alni* after two winter seasons with different climatic course, and (2) to verify the presumption of the pathogen sensitivity to the frost in vitro.
The winter survival of the invasive pathogen *Phytophthora alni* subsp. *alni* in black alder stems was studied after two very different winter seasons: the extremely mild winter in 2006/7 with an average temperature of 2.54 °C and the standard one in 2008/9 with an average temperature of –1.96 °C (the difference in temperature resembles the expected climate change in Central Europe in this century). The study was carried out in a bankside alder stand of the Moravská Dyje River (southern Bohemia). The isolation of the pathogen from the innerbark tissues of stems was conducted during the first week in the spring 2007 and 2009 and the rate of pathogen survival in the samples was ascertained. In total, 230 samples were processed. *In vitro test.* Ten tested isolates of *P.alni* subsp. *Alni* were cultivated on CA plates (25 ml medium per Petri dish) at 20 °C in the dark. The agar plugs (0.5 cm diam.) with well developed mycelium and oospores were taken from margins of two-week-old colonies. Then the plugs (with mycelium downwards) were put in regular intervals onto V8A plates (25 ml per Petri dish) and exposed to the frost. The sensitivity to the frost was tested by incubation over a range of temperature (–0.1, –2.5, –5.0, –7.5 and –10.0 °C) and frost duration (0, 7, 14, 21 and 28 days). There were incubated ten CA plugs on V8A plate in each treatment combination (isolate × temperature × frost duration). After the exposure to the frost the V8A plates were incubated at 20 °C in the dark and the number of colonies emerging around plugs (survived particles) was counted. The data were analyzed in Statistica 8.0.
Outcomes. Field observation. The significant difference ($P \ll 0.001$) of the pathogen survival in the two tested winter seasons was found out. The pathogen survived the mild winter 2006/7 much better than the cold one: it was successfully isolated from 86.09% samples with an average survival rate 25.52% (comparing to 13.91%, and 2.70% respectively). Moreover, the thickness of the covering tissues and exposure to the most heated southwestern quadrant of stem girth positively affected pathogen survival. *In vitro test.* The influence of temperature, frost duration and their interaction on *P. alni* subsp. *alni* viability *in vitro* was experimentally confirmed ($P < 0.01$). The temperature –0.1 and –2.5 °C had no significant effect on the viability however the incubation lasted for four weeks. The pathogen viability significantly decreased ($P < 0.01$) when the

temperature –5.0 °C persisted at least one week**.** At –10.0 °C no isolate survived till one week. The important differences in viability of particular isolates were found out between –2.5 and –7.5 °C.
The outcomes indicate that the frost can influence the pathogen survival in frozen aerial plant tissues and in upper layers of soil or water at least in Central, Northern, and Eastern Europe with hard continental winters. Very likely, the global warming can enhanced the impact of the pathogen on alders by its higher survival rate.

An Assessment of the Early Effects of Climate Change on Forest Health

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The current warming trend is expected to result in species migration northwards in latitude and upwards in elevation. Colonization of higher elevations and latitudes has been documented elsewhere, but a warmer climate may also increase mortality at the lowest latitude and elevation of species ranges. While local conditions may mediate some of the effects of global climate change, the trend should be detectable at large geographical scales.
Given tree size and longevity, mass mortality in the warmer boundary of species distributions may not be apparent at the early stages of warming. However, pathogen damage may increase, because trees are less capable of resisting pathogens when growing under limiting conditions. So, under a global warming hypothesis, trees at the receding edge of the species habitat must be the first to suffer disease outbreaks.
In order to test this hypothesis, we used data from the forest inventory of Washington, Oregon and California (Forest Inventory and Analysis Program, Forest Service, Department of Agriculture, USA) to study whether there was a pattern of higher disease damage associated with southern latitude and lower elevation of a species range, and with SW aspects. The area under study covers 35 million ha. of forestland across a wide range of environmental conditions. A total of 14,000 plots, 45 pathogens and 37 tree species were included.
The results confirm the presence of damage at the hypothesized sites at regional scale, but also indicate a more complex pattern. The damage is pathosystem dependent, and there seem to be some fundamental differences between diseases and insect attacks. These results would be useful to modelers in order to refine the projected effects of climate change on future tree distribution.

Himalayan Dwarf Mistletoe (*Arceuthobium minutissimum*) and the Leafy Mistletoe *Taxillus kaempferi* on Blue Pine (*Pinus wallichiana*) in Bhutan

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Blue pine, *Pinus wallichiana*, is an ecologically and economically important tree species in temperate conifer forests at elevations between 2100 and 3100 m asl. in Bhutan. Two parasitic flowering plants, Himalayan dwarf mistletoe, *Arceuthobium minutissimum*, and the leafy mistletoe, *Taxillus kaempferi* are common on Blue pine in this Himalayan country. *Arceuthobium minutissimum* is widespread and very damaging in dry Blue pine forests in the districts Paro, Ha and Thimphu in Western Bhutan. *Taxillus kaempferi* occurs most frequently on Blue pine, but also infests *Tsuga dumosa* and *Picea spinulosa*. It occurs in the districts Thimphu, Wangdi Phodrang, Trongsa, Bumthang and Mongar.
A survey in a 156-hectare-large area of Blue pine forests in the district Thimphu in Western Bhutan, conducted in 2004, has documented high infection levels of *A. minutissimum* and *T. kaempferi* on *P. wallichiana*. *A. minutissimum* occurred on 58 %, *T. kaempferi* on 52 % and both mistletoes on 30 % of the 97 sample plots. Of the 2282 Blue pine trees evaluated, 29.4 % were infested with *A. minutissimum* and 4.9 % with *T. kaempferi*, with both species occurring on 1.5 % of the trees. Incidence of both mistletoes increased with diameter of the host trees. However, *A minutissimum* was also prevalent on small trees, exemplified by the smallest diameter class (0.1 to 5.0 cm diameter at breast height), in which approximately 25 % of the trees were infected.
Forest management practices in Bhutan have greatly favoured infestation of *P. wallichiana* with *A. minutissimum* and *T. kaempferi*. This is because it is common practice to preferentially cut uninfected trees with good wood quality and leave infested residual trees during stand entries. We recommend incorporating principles of disease management, particularly sanitation in the silvicultural system to treat Blue pine forests heavily affected by these parasitic plants in Bhutan.

Dieback of Ash (*Fraxinus excelsior* and *Fraxinus angustifolia*) in Eastern Austria: Disease Development on Monitoring Plots from 2007 to 2010

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Ash dieback caused by *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*) was first recorded in Austria in 2005 and has since then become the most important damaging factor of hardwood trees. Both *Fraxinus excelsior* and *F. angustifolia* are affected by the disease. Here, results of an ash dieback monitoring project in the province of Lower Austria are presented.
In 2007, 50 monitoring plots (48 with *F. excelsior* and two with *F. angustifolia*) were established in various parts of Lower Austria. On each plot 20 mature ash trees were selected. Using binoculars, on each sample tree the percentage of crown volume affected by dieback was visually estimated in 10 % classes. Likewise, various other diseases and insect pests were recorded. In this year, ash dieback was significantly less intensive in the plain and dryer eastern parts of the province than in the mountainous and more humid western parts. In addition, suppressed individuals showed higher mean dieback intensity than dominant ash trees, and female trees were more severely affected than male ones. In almost every monitoring plot trees without symptoms were observed. From 2008 onwards, annual monitoring was continued on 15 selected permanent plots (14 with *F. excelsior* and one with *F. angustifolia*) with 20 sample trees each. In 2010, mean dieback intensity on these plots varied between 2 % and 38 %. Mortality was still very low in 2010. Dead ash trees (maximum 5 %) occurred on only three of the 15 plots. Although dieback intensity generally increased from 2007 to 2010, on some sites disease intensity did not change much and remained at low levels. During the entire observation period most of the sites in the eastern parts of Lower Austria remained less severely affected than those in the western parts of the province. Differences in relation to gender or social position were not observed anymore. Male and female individuals showed similar levels of disease intensity, as did suppressed and dominant trees. In 2010, there were still trees showing no symptoms of dieback in 13 of the 15 plots; their proportion varied between 5% and 80%. Among secondary damaging factors, ash bark beetles (mainly *Leperisinus varius*) were found on about one third of the plots already in 2007, but until 2010 there were no records of bark beetle attacks on living trees. Differences in intensity of ash dieback between sample plots and relations between disease intensity and site factors, climate as well as inoculum potential are discussed.

Mycosphaerella dearnessii M. E. Barr (Brown-Spot Needle Blight of Pine) in Austria

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Mycosphaerella dearnessii M. E. Barr (syn. *Scirrhia acicola*; anamorph: *Lecanosticta acicola* Thüm, syn. *Septoria acicola*) is an ascomycetous pine needle pathogen and the causal agent of brown spot needle blight. Potential hosts comprise various pine species and even *Picea glauca* can be infected when exposed to heavy spore loads. The disease is known from North, Central and South America, Asia, South Africa and Europe. Since it is widespread in North and Central America, it is assumed to be of Central American origin. The global spread of the fungus is attributed to the expanded pine trade in the last decades. In Europe *M. dearnessii* is mostly limited to local sites and often occurs on Mountain pine (*Pinus mugo)* and Scots pine (*Pinus sylvestris)* in urban habitats (parks, gardens) as well as in arboreta. In forests *M. dearnessii* is known to occur on *Pinus uncinata* in swamps and more rarely in *Pinus sylvestris* / *Pinus radiata* stands.
In Austria, brown spot needle blight was identified originally from Mountain pine (*Pinus mugo*) in 1996 in the town Hollenstein/Ybbs (Lower Austria). Annual surveys by the Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW) from 1996 to 2007 revealed a slowly increasing number of infested trees (*Pinus mugo, Pinus uncinata* and *Pinus sylvestris*) but infestation was still limited to urban sites in that town. In August 2008, however, the species was found for the first time in mixed forest stands on Scots pines (*Pinus sylvestris*) adjacent to the town of Hollenstein. In autumn 2009 newly infested trees were found at the border of the municipal area of Hollenstein close to further mixed pine forest stands. In autumn 2010 *M. dearnessii* was detected for the first time on urban trees in five other towns up to 40 km distant to Hollenstein.
Austrian black pine (*Pinus nigra austriaca)* which is common in the town of Hollenstein and known to be susceptible was never infected by *M. dearnessii.* This point might be due to a probable competition effect between *Dothistroma septosporum* (Dorog.) M. Morelet, which is extremely common on this pine species in Austria, and *M. dearnessii*. Swiss stone pine (*Pinus cembra*) which was planted as an ornamental tree in Hollenstein was also never infected.
Whether the disease spread naturally from Hollenstein to the other towns, or infections were the result of multiple introductions (via infested plant material?) is subject of discussion. Pathogen spread can occur over short distances by rain splash and wind, however, it is also likely that spores can be transported by clothes, shoes, or vehicles. Heavily infested trees, suffering from intense needle losses for many years, show branch dieback extending upwards in the crown. It is very likely, that such trees become attacked by secondary invaders.
Currently, a doctoral thesis is conducted at the University of Natural Resources and Life Sciences (BOKU), Vienna and the BFW. One goal of this thesis is to investigate population diversity and potential patterns of spread of *M. dearnessii* in Austria as well as genetic diversity of *M. dearnessii* in Europe and other continents.

Alien Pathogens of Forest Trees in Austria

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Alien pathogens are an important threat to the world's forests and the various ecosystem services they provide to humans. In recent years there have been various initiatives to synthesize information on alien organisms in general and forest pathogens specifically. Based on a literature survey and own records we present a synthesis of alien pathogens occurring on forest trees in Austria. Pathogens were classified either as definitely or ambiguously alien, the latter including those species for which it is not precisely known, whether they are non-native or not.

A total of 29 pathogens of forest trees were recorded for Austria, 15 of which being definitely alien and 14 whose status is ambiguous. The Austrian list of alien and possibly alien forest pathogens includes one bacterium (*Erwinia amylovora*), nine *Phytophthora* species and 19 true fungi. Amongst fungi, ascomycetes dominate with 14 species, three are anamorphic fungi and two are basidiomycetes, both being rust fungi. For 12 out of the 15 definitely alien pathogens, the continent of origin is known. Seven are native in North America and five in Asia. Special cases are *Phytophthora alni* that originated from hybridization of two unknown *Phytophthora* species and *Cronartium ribicola* that is native in the Alps, but the epidemic on *Pinus strobus* was likely caused by strains of the fungus introduced from Asia.

Amongst conifers native to Austria only pine species (*Pinus* spp.) have been affected by two alien and one ambiguously alien pathogen. Six pathogens occur exclusively or mainly on non-native tree species and 21 utilize various native deciduous tree species as hosts. While the ecological and socioeconomic impacts of most pathogens have been or are presently considered as low, six species that cause serious diseases and can lead to tree killing have been particularly problematic. These include *Ophiostoma novo-ulmi* (on *Ulmus* spp.), *Cryphonectria parasitica* (on *Castanea sativa*), *Cronartium ribicola* (on *Pinus strobus*), *Phytophthora alni* (on *Alnus glutinosa* and *A. incana*), *Diplodia pinea* (mainly on *Pinus nigra* in Eastern Austria) and *Hymenoscyphus pseudoalbidus* (on *Fraxinus excelsior* and *F. angustifolia*). Five of these particularly damaging forest pathogens have appeared during the last 60 years, corresponding to one serious emerging disease caused by a definitely or ambiguously alien pathogen occurring on an average every 12 years.

Ash Dieback in Forest Nurseries in Austria

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During the last two decades ash dieback caused by the ascomycete fungus *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*) has successively emerged in many European countries. Everywhere the disease appears it causes immense damage to common ash (*Fraxinus excelsior*) and locally also to narrow-leaved ash (*Fraxinus angustifolia*). Trees of all ages, in the forest, the landscape and in urban environments, both naturally regenerated and planted ones are affected. Moreover, ash dieback is an economically important problem in shade tree and forest nurseries.
In 2008 and 2009 we did investigations on ash dieback in four forest nurseries in Austria. Typical symptoms of the disease, including necrotic bark lesions as well as dieback of shoots and entire seedlings were frequently observed in all four nurseries, on one-year-old to three-year-old seedlings. From seedlings showing early disease symptoms, fungal isolation was attempted. After surface sterilization (1 min 96 % ethanol, 3 min 4 % NaOCl, 30 s 96 % ethanol) of stem segments, the outer bark was carefully peeled off and small discs containing wood and phloem were cut near the edge of the necrotic phloem lesion and placed on malt extract agar (20 g/l malt extract, 16 g/l agar, 100 mg/l streptomycin sulphate). *Hymenoscyphus pseudoalbidus* was consistently isolated at high frequencies and often in pure culture from *F. excelsior* seedlings in all four nurseries and in one nursery also from *F. angustifolia* seedlings. Overall, the ash dieback pathogen was isolated from 142 of the 160 (89 %) investigated *F. excelsior* seedlings and 14 of the 15 (93 %) examined *F. angustifolia* plants. In agreement with a number of recent studies, the results clearly suggest that *H. pseudoalbidus* is associated with ash dieback.
Hymenoscyphus pseudoalbidus is effectively disseminating and spreading via its airborne ascospores. However, infected nursery seedlings could have been and still may be an important pathway to introduce the pathogen into new areas and to accelerate its spread in Europe. Plant quarantine measures for nursery seedlings may be effective to avoid or delay the movement of the ash dieback pathogen to geographically isolated parts of Europe such as the British Isles and to other continents, where susceptible host species occur. In this presentation, we will also give recommendations for disease management in tree nurseries.

Ophiostomatoid Fungi Associated with the Eastern Himalayan Spruce Bark Beetle (*Ips schmutzenhoferi*) in Bhutan: Species Assemblage and Phytopathogenicity

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The Eastern Himalayan spruce bark beetle, *Ips schmutzenhoferi* is an important pest of *Picea spinulosa* and *Pinus wallichiana* in temperate conifer forests in the Bhutan Himalayas. In 2001, we conducted a survey of ophiostomatoid (blue-stain) fungi associated with this conifer bark beetle in western Bhutan. Eleven species were found to be associated with *I. schmutzenhoferi*. These included *Ceratocystiopsis minuta*, *Ceratocystis bhutanensis*, *Grosmannia* cf. *cucullata*, *Ophiostoma* cf. *ainoae*, *Leptographium* sp. 1 (associated with a *Grosmannia* teleomorph), *Leptographium* sp. 2 and five taxa in the *Ophiostoma piceae* species complex. The latter comprised *O. floccosum* (*Pesotum aureum*), *O. quercus*, *Ophiostoma* cf. *piceae*, *O. setosum* (*Pesotum cupulatum*) and *Pesotum* cf. *quercus*. *Leptographium* sp. 1 appeared to be the dominant fungal associate of *I. schmutzenhoferi* and *O.* cf. *ainoae* was also common.
In 2005, two isolates each of *C. bhutanensis, Leptographium* sp. *1, O.* cf. *ainoae,* and O. cf. *piceae* were used in a pathogenicity trial on *P. spinulosa* and *P. wallichiana*. All four fungi caused only small necrotic lesions in the phloem of *P. wallichiana* trees, with average lesion lengths ranging from 18 to 28 mm. *Ceratocystis bhutanensis*, *O.* cf. *ainoae* and *O.* cf. *piceae* also caused relatively small lesions on *P. spinulosa* (range of average lesions lengths: 30 to 34 mm). In contrast, Lepographium sp. 1 incited very long phloem lesions on *P. spinulosa*, averaging 223 and 296 mm for the two isolates. In a mass inoculation experiment *C. bhutanensis* displayed a very low level of virulence to *P. wallichiana* and a moderate level of virulence to *P. spinulosa*.
Our studies in Bhutan have shown that *I. schmutzenhoferi* is intimately associated with ophiostomatoid fungi. The most common fungal associate of this bark beetle species, *Leptographium* sp. 1 displayed high levels of virulence to *P. spinulosa*. Moreover, the pathogenicity trials suggest that fungal associates of *I. schmutzenhoferi* and especially *Leptographium* sp. 1 prefer *P. spinulosa* over *P. wallichiana* as host, as is true of the insect itself.

Chalara fraxinea Incidence in Hungarian Ash (*Fraxinus excelsior*) Forests

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Chalara fraxinea was identified for the first time in Hungary in the first half of 2008, in western Hungary near Kapuvár and Sárvár, in 4-6 years old mixed (seed and coppice shoot) origin European Ash (*Fraxinus excelsior*) stands. In the same time in Budapest, under an older Turkey Oak – Sessile Oak – European Ash stand we also detected the symptoms and the pathogen on the saplings of the natural regrowth. The local foresters first thought, that the wilting was caused by the frost, but in that period of time there were no frosty days. With the typical symptoms and the examinations of the collected samples, we were able to definitely identify the *Chalara fraxinea* as pathogen. This pathogenous fungus was also identified on Narrow-leafed Ash (*Fraxinus angustifolia*), from the samples of the western part of Hungary. In 2008-2009 we thoroughly researched the distribution of the pathogen in Hungary, and the volume of the caused damages. As a result, we confirmed, that the pathogen spread to the whole area of Hungary. It appears both in young and older stands, but it causes damages more frequently in 2-10 years old forestations. Because of the characteristics of the symptoms and the measures of the dieback, we concluded, that the pathogen appeared in Hungary 2-3 years before. The degree of the infections in the examined forest stands is significantly diverse. The most severe infestation was observed in Eastern-Hungary, near Debrecen, in the summer of 2009. This European Ash stand was 10 years old, with 0,5 ha of area, and was planted with 2 years old saplings. Every single tree showed the symptoms of *Chalara fraxinea* infection. In the examined part of the forest-stand, the mortality reached 37%. Among the still living trees, the rate of the infected and died stem parts varied between 20-90%. From the symptoms of the dead trees we diagnosed, that the first infections in this area also occurred a few years ago. We do not know much about the environmental conditions assisting the infestation. The examinations of the infested forest-stands of Western-Hungary shows, that the infestation is more frequent on sites with frost-hollow, deep soil and plenty of water. In the same time we also noticed, that the symptoms are also frequent on forest sites drier than average and exposed to extreme cold. According to the surveys, the fungus is more common in younger stands, but this can be affected by the fact, that we have lesser amount of samples from older and bigger trees, for collecting samples and identifying them from large crowns is more difficult. After the survey in Bükk-mountains, North-eastern-Hungary, we found that the extent of the infection is at least the same on older or middle aged trees, than on the youngest ones. Contrarily, in the western part of the country in mixed species forest stands we experienced mass and severe infections of the natural Ash regrowth, while older trees showed only small degree of typical symptoms in their crowns. The complete death of older trees takes more time, so major mortality occurs on young, 2-10 years old trees.

In the august of 2009 we surveyed the degree of *Chalara fraxinea* infestations in some forestry's of the Bakony-mountains, in different aged and in different tree-species composition forests. Based on this survey we pointed out, that in the significant majority of the surveyed stands the rate of infected ash trees is under 5%, and in only 2 forest-parts are there 5-10% infestations.

To summarize our researches so far, it seems that in Hungary the European and Narrow-leafed Ash forests are seriously endangered by *Chalara fraxinea*, especially the young stands. The results of the extended life-cycle examinations of this pathogen are indicating that we are defenceless against the pathogens infestation; we cannot effectively control the pathogen or decrease the severity of infestations. In the future presumably natural selection will work among Ashes, which will seriously affect us with mass mortality of trees. In the same time it is our task to assist these processes with the selection of more resistant tree individuals, and with the mass propagation of these samples using them in forestry practice.

Phytophthora-Infection in a Sweet Chestnut (*Castanea sativa*) Orchard in Transdanubia, Hungary

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In the first decades of the 20th Century, a big amount of the sweet chestnut (*Castanea sativa*) stands in South-Europe died. The cause was the ink disease. Since then, the ink disease is one of the most serious diseases of the sweet chestnut in whole Europe. There are several *Phytophthora* species in contact with the appearance of the ink disease. The most frequent species are *Phytophthora cambivora* and *Phytophthora cinnamomi*. The appearance of ink disease is sparse in Hungary.

In September 2010, we have found dying trees in a sweet chestnut orchard of 14 year old in South-Transdanubia, Hungary. The symptoms were specific: small, yellowish leaves, sparse, drying foliage, necrotic bark lesions at the stem basis and main roots. The fine roots were rot. Some saplings also died in the same orchard.

We have isolated *Phytophthora* species on selective agar media from soil samples taken from the rhizosphaere of the dying trees and saplings by baiting with *Rhododendron* leaves. The species identification was carried out by morphologic examination and the ITS1 and ITS2 sequences of the ribosomal DNA. We identified *Phytophthora cambivora* from the soil of dying trees and of saplings, too.

Now, the appearance of ink disease in the orchard is confined to a small clump. It is an urgent task to find the adequate methods to confine the disease.

The Ash Dieback Pathogen *Hymenoscyphus pseudoalbidus* is Associated with Leaf Symptoms on Ash Species (*Fraxinus* spp.)

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Ash dieback, an emerging infectious disease of common ash (*Fraxinus excelsior*) and other *Fraxinus* species in Europe, is caused by the ascomycete fungus *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*). Besides necrotic lesions in the phloem and wood discoloration leading to dieback of shoots, leaf symptoms have been related to this disease. These symptoms include necrotic lesions on leaf petioles and rachises as well as leaflet veins, often followed by wilting and early leaf shedding. Fungal isolation in 2008 and 2009 has shown that *H. pseudoalbidus* is consistently associated with necrotic lesions on leaf rachises of *F. excelsior*.

In June 2010 leaf rachises of potted *F. excelsior*, *F. angustifolia* and *F. ornus* seedlings were wound-inoculated with five *H. pseudoalbidus* isolates. Inoculum consisted of autoclaved wood fragments (approximately 5 mm long and 2 mm in diameter) obtained from *F. excelsior* shoots that had been placed for 24 days on the various *H. pseudoalbidus* cultures on malt extract agar (MEA). Sterile wood fragments of similar size were used as control treatment. Inoculation was done by initiating an approximately 1-cm-long superficial wound on the upper surface of a leaf rachis, placing inoculum on the wound and fixing it with parafilm. For *F. excelsior* and *F. angustifolia* 20 plants per treatment were inoculated, while for *F. ornus* 10 seedlings per treatment were used. After inoculation the seedlings were regularly inspected for leaf symptoms until early November. Upon dropping of an inoculated leaf from a seedling or at the termination of the experiment, re-isolation of *H. pseudoalbidus* from the leaf rachis onto MEA was attempted.

Leaf symptoms (necrosis, wilting and dropping) appeared on all three ash species tested. Some inoculated leaves showed typical symptoms already two weeks after inoculation. The temporal patters of leaf shedding varied considerably between the three ash species. For example, five weeks after inoculation only one fungus-inoculated *F. ornus* leaf had dropped, whereas the proportions of shed leaves were more than 50 % in *F. excelsior* and 11 % in *F. angustifolia*. Necrotic lesions on the leaf rachis developed on 91 % of the *F. excelsior* seedlings, 56 % of the *F. angustifolia* seedlings and 53 % of the *F. ornus* seedlings inoculated with *H. pseudoalbidus*. The values for the occurrence of leaf wilting were 74 % (*F. excelsior*), 29 % (*F. angustifolia*) and 30 % (*F. ornus*). Besides a few natural infections, no symptoms occurred on the control plants. *Hymenoscyphus pseudoalbidus* was re-isolated from all three *Fraxinus* spp., often in pure culture, but results varied between species. Re-isolation rates from fungus-inoculated seedlings were 64 % for *F. excelsior*, 53 % for *F. angustifolia* and 27 % for *F. ornus*.

Kochs postulates were fulfilled to conclude that *H. pseudoalbidus* is responsible for the leaf symptoms on *F. excelsior*. Likewise, in the inoculation experiment the ash dieback pathogen caused leaf symptoms on *F. angustifolia* and also on *F. ornus*. The results support the view that leaf infections are important elements of the disease cycle of ash dieback. Leaves may be the most important path of *H. pseudoalbidus* to infect shoots of *F. excelsior* and other ash species.

Seasonal Variation in the Infection Level of *Cedrus libani* Needles by *Ploioderma cedri*

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Cedrus libani forests are presently found mainly in the Taurus Mountains of Turkey while only small populations of the once extensive and magnificent cedar forests remain in Lebanon and Syria. Since 2004, we have observed browning of needles in spring in the lower part of the canopy of the trees in some *C. libani* stands in the lakes district of Turkey. The disease has occurred both on saplings growing as understory in mixed forest as well as on approximately 10–m–tall trees in an even–aged stand. The frequent fruiting of *Ploioderma cedri* on dead parts of otherwise green needles indicates that the fungus was the causal agent of the disease. There has been considerable variation in the level of infection from one year to another. In the present study, we estimated the effect of the disease on needle biomass.

Powdery Mildew Fungi on some Deciduous Tree Species in Turkey

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Powdery mildew is a quite common disease that appears as a white powdery substance occurring on the leaf surface, stem and fruits of many deciduous tree species in European forests. As a result of heavy infection leaves and affected parts of the tree frequently are distorted and in worst cases infected trees may exhibit symptoms of defoliation or decline. In this study some deciduous tree species such as *Platanus orientalis Quercus vulcanica, Quercus robur, Castenea sativa* were inspected for the occurrence of powdery mildew. Additionally, infection rates and distribution were studied from the visible symptoms of the disease observed on *P. orientalis* saplings from seed orchards of Çınarcık, Yalova.

Q. vulcanica which is an endemic oak species of Turkey shows disease symptoms every year in Kasnak Oak National Park in Isparta Province together with *Q. robur*. *C. sativa* has also powdery mildew infections observed abundantly in one of the recreation areas in Isparta. As a result of macroscopic and microscopic studies several different fungal species causing powdery mildew on these hosts were found. Mainly characteristics of cleistothecia were used for the identification of the powdery mildew fungi. While *Microsphaera alphitoides* was common fungus on the upper surface of *Q. vulcanica* and *Q. robur*, only *Phyllactinia roboris* which is known as a rare species was detected on the lower surface of the leaves of *Q. vulcanica*. Sweet chestnut and plane trees were infected by *Phyllactinia guttata* and *Microsphaera platani*, respectively.

The infection rate and the distribution of *M. platani* on 100 *P. orientalis* saplings were investigated. The saplings were approximately 2.5 cm in diameter and 160 cm in height. In each tree three topmost lateral shoots and the terminal shoot were checked. All leaves of each shoot were counted and investigated for the presence of the fungus. While all *Platanus* seedlings were found to be infected with *M. platani* the disease was more common on terminal shoots (85.8%) than on the lateral ones (52.4%).

Red Band Needle Blight – Molecular Screening of the Czech Republic

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Red band needle blight is considered to be a serious disease of pine trees and some other conifers. It is caused by two cryptic deuteromycete species, *Dothistroma septosporum* (teleomorph *Mycosphaerella pini* Rostr.) or *Dothistroma pini* (teleomorph unknown). Originally considered one species, *D. septosporum* and *D pini* were distinguished in 1993 by Barnes using morphological a molecular markers. Identification just by morphological charactistics is not easy and in most cases even impossible. Using specific primers designed by Ioos (2010), screening and differentiation of these two species is feasible. The entire collection of *Dothistroma* strains of the Department of Forest Protection and Wild Life Management was screened. DNA of *Dothistroma* specimens was isolated using commercially available kits, the specific recognition regions were analyzed using PCR and and visualized on agarose gels.

Antagonistic Effect and Reduction of *Ulmus minor* Symptoms to *Ophiostoma novo-ulmi* by Elm Endophytes

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Ophiostoma novo-ulmi is the causal agent of Dutch elm disease (DED), which has developed into one of the most devastating forest diseases. Although often ignored, endophytic symbionts may play an important role in plant fitness, protecting the plant against biotic and abiotic stressors. In the present study, we have isolated endophytic fungi from bark and xylem tissues of healthy *Ulmus minor* trees from Spain. Several elm populations in Majorca Island and a clone collection in Madrid, including elm genotypes with high resistance level to DED, were sampled. The interactions between 31 endophytic fungi and two *O. novo-ulmi* strains were in vitro tested by means of dual plate assays. Three types of interactions were evaluated: antibiosis, competition for substrate, and mycoparasitism. Endophytes won the interaction against *O. novo-ulmi* at 58% of the dual tests, mostly by competition for substrate. The pathogen won the interaction at 26% of the cases, and the interaction was considered neutral at the remaining 16%. Four endophytic strains were selected on the basis of their strong antagonistic effect against *O. novo-ulmi*, the resistance level to DED of their hosts, and the tree organ from where they were isolated. In order to evaluate in vivo their protective effect, these four strains were inoculated in 4- to 8-year-old *U. minor* plants 10 days before challenge with *O. novo-ulmi*. Three of the four selected endophytes significantly reduced DED symptoms with respect to control plants ($P < 0.05$). Although more evidence is needed to fully evaluate the potential of endophytes in biocontrol of DED, these results open new prospects to elucidate the functional roles of endophytes in forest ecosystems and to develop future strategies for sustainable disease management.

Endophyte communities associated with northern Spain forests: influence of environmental variables

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Over the 60´s a lot of pine plantations were established in the north of Spain, coexisting with native forests of *Quercus pyrenaica* Willd. Nowadays, they need to be conserved because of their ecological relevance. For this purpose, it is necessary to know thoroughly the existing relationships among members of forest systems. Fungal endophytes, which colonize living plant tissues without causing any immediate overt negative effects, are still poorly known, especially in the Mediterranean region. Furthermore, it is also important to study the effect of different environmental and silvicultural variables on fungi.
Branches and needles/leaves of *Pinus sylvestris*, *P. nigra*, *P. pinaster* and *Q. pyrenaica* from 36 trees (in twelve sites, three per species) of the province of Palencia were collected. Thus, environmental, dendrometric and dasometric features of the host were measured in order to find a relation with respect to the fungal endophytic community. A total of 46 fungal species were isolated, and some relations with variables studied were found.

Monitoring Damage from Foliage, Shoot and Stem Diseases in New England and New York

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Forests are an important resource in New York and the six States that comprise New England. Sixty-six percent (206 646 km^2) of the land area is forested, 83 percent of which is privately owned. The region's forests are ecologically diverse. The most common forest types are maple (*Acer* spp.), spruce/fir (*Picea*/*Abies* spp.), other hardwoods (*Fagus*, *Prunus*, *Fraxinus*, *Betula*, *Carya*, *Quercus* spp.), hemlock (*Tsuga canadensis*) and pine (*Pinus* spp.). Foliage, shoot, and stem diseases are common in the region due to favorable climate and the wide variety of available host tree species. To protect forest resources, State forestry agencies in cooperation with the US Forest Service monitor forest health conditions annually. States participate in national aerial detection surveys and visit resource-specific and pest-specific permanent plots. Data from these surveys are analyzed to assess impact of forest diseases and insect pests. For instance, data from aerial and ground surveys are used to map the spread of beech bark disease, which is endemic in New England and New York but continues to expand throughout the range of American beech. Beech bark disease is a disease complex involving the beech scale (*Cryptococcus fagisuga*) and canker fungi in the genus *Neonectria*. Aerial and ground surveys are also used to identify forest disease trends. For example, foliar diseases and shoot blights including anthracnoses, conifer needle casts, and *Sirococcus* shoot blights proliferated since at least 2006 due to unusually wet spring weather. Results from forest health monitoring efforts are made publicly available via web-based products such as the Forest Pest Portal (http://www.foresthealth.info/) and Forest Health Highlights (http://fhm.fs.fed.us/fhh/fhmusamap.shtml).

Epidemic and Pathogenicity of *Chalara fraxinea* Causing Ash Dieback in Hungary

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In Hungary the ash dieback caused by *Chalara fraxinea* was first observed in spring 2008 in the plots of the Sárvár and South-Hanság Forestry Management units.
The disease affected both artificially planted saplings and natural regrowths. Symptoms included leaf and shoot wilting, brown discolouration in the bark as well as greyish-brownish discolouration of the wood.
Since then the disease has been proved to be widespread and to endanger the health state of ash trees of different age seriously.
The susceptibility of ash species was examined by artificial infection of one-year-old saplings in a nursery. More hundred saplings were wound-inoculated with the mycelium of the pathogen.
Common ash and narrow-leafed ash were found susceptible in the experiment: 24 and 21 % of the inoculated trees showed wilting after 2-3 weeks. The green ash and the flowering ash did not alter.
Between 2008 and 2010 investigations were carried out in an artificial common ash regeneration. The frequency of the symptoms and their dinamic were observed in three survey plots of 0.1 hectare each.
In 2008 the frequency of the symptoms of the disease was low, only 0.8 – 1.2 %. In 2009 the disease spread significant: 8.2 - 20.9 % of the trees showed fresh symptoms. In spring 2010 the number of infected trees decreased again: 2.7 - 9.3% of the shoots wilted. This change is connected with the lower amount of rainfall in summer and autumn of the previous year.
The precipitation in the infectious period (August-September) should determine ash dieback in spring next year.

REINFFORCE (REsource INFrastructure for monitoring and adapting European Atlantic FORests under Changing climatE) "Establishment a Network of Arboretums and Demonstration Sites to Assess Damages Caused by Biotic and Abiotic Factors"

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The reality of global warming is recognized worldwide, and most of the climatic models in the optimistic scenarios of IPCC, predict a 4ºC temperature rise over the next 50 years. Nevertheless, the regional consequences are still unpredictable, especially in the case of ocean areas, because there are many unknown factors like the climatic, economic and environmental conditions at regional level. However, some specific threats are appearing such as, disturbances in the life cycle of tree species, the introduction of new pathogens, or the misadaptation of tree species to new climatic conditions. The European Union Project (INTERREG IVB) REINFFORCE offers the opportunity to install a network of arboretums and demonstration sites unique in the world, located between latitudes 37° and 58° for monitoring the adaptation of European Atlantic forests to climate change through the study of the tree growth, its phenology and the forest health. The participants belong to eleven institutions from United Kingdom, France, Spain and Portugal. This is a key issue for sustainability of Atlantic forest resources, as the trees that are now being planted, will be harvested in 50 years facing new climatic conditions.

The goals of the REINFFORCE project are: i) to establish protocols for the installation of infrastructures and data collection; ii) to perform the technical and administrative evaluation of the work; iii) to create a network of arboretums to anticipate the effects of climate change; iv) to implement a network of demonstration sites to compare usual silviculture with other adaptative measures; and v) to develop databases to share online.

To achieve these objectives, The University of Valladolid is responsible to create, manage and explore two arboretums and two demonstration sites located in Cantabria and three arboretums and two demonstration sites located in Castilla y León. Thirty one tree species with 3 to 9 provenances of each species are going to be tested in these arboretums. On the other hand, the demonstration areas are mixed plantations based on Nelder wheels to evaluate the influence of the density on the mortality, the size, the pathogenic damages and the biomass allocation of each plant. Protocols to measure growth parameters, to study the phenological aspects, and to assess biotic and abiotic damages, are being developed for all of these infrastructures.

Evaluation of Genetic Resistance to *Fusarium circinatum* in *Pinus* Species

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Pitch canker is a disease caused by the fungal species *Fusarium circinatum.* This disease is the main cause of damages in nurseries and plantations of *Pinus* in the northern area of Spain. Its presence involves applying eradication measures producing serious economical, ecological and social impacts in the Cantabrian coast. Susceptibility to these pathogens could be due to a variety of factors such as drought, physical damage or other environmental stresses, and host species. Currently, control, prevention and eradication of *Fusarium circinatum* are hard to achieve and disease management becomes difficult and highly expensive.
The objective of this study was to evaluate the response of the main conifer species grown in Spain, *Pinus sylvestris* L., *P. nigra* Arnold, *P. pinaster* Aiton, *P. radiata* D. Don, *P. halepensis* Mill, *P. pinea* L. and *P. uncinata* Mill. Ex Mirb. to the inoculation of 5 isolates of *Fusarium circinatum* (Mat 1 and Mat 2). Artificial inoculations are considered to be a convenient and relatively effective way of evaluating the inter-specific resistance of pines to *Fusarium circinatum*. Accordingly, two-year-old shoots were inoculated with a drop of spore suspension placed in a wound previously done. Lesion length was measured three weeks after inoculation. The experiment design was a completely randomized factorial. Analysis of variance and multiple comparison procedure of Bonferroni was performed on the lesion length. Preliminary results show that *Pinus radiata* is the most susceptible species to *F. circinatum*, whereas the most resistant are *P. pinea*, *P. halepensis*, *P. nigra* and *P. pinaster*. Mat 2 isolates were more virulent than Mat 1 isolates.

Investigations on *P. plurivora and P. pini* in Finland

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The ornamental plant trade has unwittingly trafficked alien Oomycetes, such as pathogens in the genus *Phytophthora*, around the globe. So far four *Phytophthora* species have been identified on seedlings in Finland: *P. cactorum, P. plurivora*, *P. pini* and *P. ramorum*. The recently described *P. plurivora* is found to be abundant in semi-natural ecosystems and nurseries across Europe, causing bark necroses, fine root losses and dieback on numerous host tree species. We have isolated *P. plurivora* (previously identified as *P. inflata*) for the first time in 2004 from rhododendron cultivars, and later from *Syringa vulgaris* in two nurseries in Southern Finland. Since the original finding, it has been found almost every year, despite attempted eradication procedures. Also *P. pini* was isolated once in 2007. *P. pini* has been found widespread in the eastern USA, causing damage and mortality to introduced species such as *Fagus sylvatica*. In Europe it has only been found in nurseries, which indicates a recent introduction to the continent. We tested the susceptibility of Finnish tree species in addition to other forest plants to infection by *P. plurivora* and *P. pini*. In our pathogenicity trials, both species were able to infect most host plants including *Fragaria* x *ananassa*, *Rhododendron* sp., *Betula pendula, Alnus incana, A. glutinosa, Picea abies, Vaccinium uliginosum, V. myrtillus, V. vitis-idaea* and *S. vulgaris*. The only resistant woody species in our trials was *Pinus sylvestris*. Both hyphae and oogonia were seen in cortical cells of Norway spruce seedlings inoculated with *P. plurivora*. In preliminary trials for control measures, none of the tested chemicals (Aliette, Restart, Cumin oil) was effective with the concentrations and application schedules used. None of the *Phytophthora* species present in Finnish nurseries has been found in natural ecosystems. As *P. plurivora* has been very recently encountered on many tree species in semi-natural ecosystems in other Scandinavian countries, further screening for this tree pathogen in addition to other *Phytophthora* spp. will be conducted in Southern Finland in 2011.

Interaction Between *Gremmeniella abietina* and Several Fungal Endophytes

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Gremmeniella abietina is the causal agent of Scleroderris canker and Brunchorstia dieback in many conifer species. It has caused severe losses in nurseries and plantations worldwide and several outbreaks have occurred in North America and Central-North Europe in recent decades. In Spain, it was first isolated from symptomatic *Pinus halepensis* trees in 1999. Since then, several studies have been conducted to either determine or to evaluate its morphological, physiological and genetic variability, pathogenicity, and potential control strategies to be used if necessary. Among those studies, the evaluation of several fungal endophytes to be used as potential biocontrol agents against *G. abietina* has been carried out. In the present communication the results of co-inoculations in the same *P. halepensis* seedling of both *G. abietina* and the fungal endophyte under greenhouse conditions are presented. Two isolates of *G. abietina* and two endophyte species, which had a previously antagonism behaviour against *G. abietina in vitro*, were used in the experiments. Inoculations were made on 1-year-old *P. halepensis* seedlings by wounding bark at 8 and 4 cm below the shoot apex. Mycelium of *G. abietina* was placed in the lower wound and mycelium of the endophyte in the upper one. Fifteen seedlings per treatment were inoculated. Additionally single inoculations with each fungal specimen were also made to evaluate their pathogenicity in *P. halepensis*. Ten weeks after inoculation the disease degree caused by *G. abietina* was evaluated by means of the necroses length caused by the pathogen. The statistical analyses showed that none of the endophytes had a significant influence on the isolates of *G. abietina*, as the necroses length caused by *G. abietina* when it was inoculated alone was in the same range of the lenght when it was inoculated with the endophyte. It was also ascertained that the isolates of *G. abietina abietina* had different aggressiveness and that endophytes were not pathogens as none of them caused a necroses length greater than the ones detected in the controls. Further studies, including different timing and inoculation techniques, would be required in order to evaluate in more detail the antagonistic effect *in vivo* of such endophytes, previously stated to have a great antagonism on *G. abietina in vitro*.

Factors Influencing Canopy Loss in Pine Stands Infected by *Gremmeniela abietina* in Northern Spain

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In this study was evaluated the influence of environmental and silvicultural variables on defoliation and leaf area index (LAI) in *Pinus halepensis* stands in Northern Spain where *Gremmeniella abietina* infections have been reported. Hemispherical photography analysis was used as an indirect method to estimate LAI, and ICP-Forest methodology was used for defoliation estimation. Multivariate and multiple regression analyses were carried out to identify the main factors influencing defoliation and LAI. The results showed that Defoliation is affected by canopy depth, age, basal area and elevation, while LAI is determined by mean diameter, density and canopy openness. The relationship between defoliation and LAI was very weak and factors affecting both parameters were different. The way in which *G. abietina* produces dieback and defoliation, being the latter heterogeneously distributed through the canopy (affecting principally the upper part of the canopy), decrease the correlation between both parameters, while for other defoliating agents, such as some insects, this relationship has been clearly proved.

Effect of Temperature on Survival of *Fusarium circinatum*

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Needles and wood pieces of *Pinus radiata* were inoculated with an isolate of *Fusarium circinatum* and placed on Fusarium-free soil in plastic containers stored at 5, 20 and 30ºC. Three replicates for each temperature and type of tree part were done. Needles and wood pieces were sampled periodically to estimate survival of *Fusarium circinatum*. This was assessed as the percentage of tree parts cultured on Selective Fusarium Agar (SFA) from which the fungus was recovered. Positive isolation was microscopically confirmed by the presence of circina growing on Spezieller Nährstoffarmer Agar (SNA) medium. *F. circinatum* was recovered from 100% of the inoculated wood pieces during the first seven and nine months at 20 and 5ºC respectively, while it decreased very slowly from the beginning at 30 ºC. After 380 days, *Fusarium circinatum* was recovered in more than 70% of the inoculated wood pieces for all temperatures tested. In needles, *F. circinatum* survived in almost 100% of the samples after nine months at 5 and 20ºC. Survival on needles at 30ºC began to decrease after three months, and it was the lowest (89%) after 380 days.

Distribution of *Mycosphaerella* Leaf Disease on Eucalyptus in Portugal

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Eucalypt plantations represent the main source of wood for the pulp and paper industry and are affected by an important foliage disease worldwide - the complex of *Mycosphaerella* and *Teratosphaeria* species *(Mycosphaerella* leaf disease). These genera affect mainly young trees with juvenile-phase foliage, causing premature defoliation, decreased growth and wood production.
Species of *Mycosphaerella senso lato* reported on eucalypts in Portugal are *T. molleriana, T. africana*, *M. walkeri, M. madeirae*, *M. communis*, *M. heimii*, *M. lateralis*, *M. marksii*, *T. nubilosa* and *T. parva.* Since 2004, in order to complete the survey, symptomatic leaves were collected from *E. globulus* plantations. Morphological and molecular characterization was used to give a clear indication of the population composition and the main species.

Cultural Detection of *Diplodia* Shoot Blight Pathogens from Red Pine and Jack Pine Seeds

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The pine shoot blight and canker pathogens *Diplodia pinea* and *D. scrobiculata* are among the many fungi associated with seed cones of conifers. Each of these species has been found to commonly and abundantly sporulate on cones of red pine (*Pinus resinosa*) and jack pine (*P. banksiana*) collected in Wisconsin and Minnesota forests. Cultural methods were used to investigate the incidence of these fungi on or in seeds obtained from government nurseries in Minnesota (three red pine seedlots) and Wisconsin (five red pine seedlots and five jack pine seedlots). Seeds were extracted, cleaned, and stored using standard methods at each nursery. In each of three replicate trials, seeds of each lot were assigned to four treatments: 1) not surface disinfested, 100 seeds; 2) surface disinfested, 50 seeds; 3) surface disinfested and then inoculated with *D. pinea* conidia, 50 seeds; or 4) not surface disinfested but then inoculated with *D. pinea* conidia, 50 seeds. Each seed was placed in a slant containing tannic acid agar and autoclaved pine needles, and incubated for up to 6 weeks. Development of pycnidia with conidia consistent with those of *Diplodia* species indicated presence of either pathogen. For red pine seeds, the mean percentage positive was 2.7% for treatment 1 and 1.3% for treatment 2. Jack pine seeds were less frequently positive for both treatments. Using species-specific PCR primers, the *Diplodia* species cultured was identified as *D. pinea* in almost every case, with identification of *D. scrobiculata* only rarely. *D. pinea* was much less frequently detected from seeds that were not surface disinfested but then inoculated (treatment 4) compared to seeds that were inoculated with *D. pinea* after surface disinfestation (treatment 3). This indicated that presence of seed-surface microflora led to underestimation of the actual presence of the pathogen in treatment 1. Results confirm the potential for dissemination of *D. pinea* on red pine and jack pine seeds. And although the frequency of positive seeds was low, the large numbers of seeds planted in nurseries suggest that seeds may be a potentially important route of entry of *D. pinea* into nursery beds.

Expansion in the Known Geographic Distribution and Host Range of the Shoot Blight Pathogen *Sirococcus tsugae*

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Eastern hemlock (*Tsuga canadensis*) is an ecologically and economically important conifer from the north-central United States to the east coast of North America to the southern Appalachian Mountains. In early spring 2010, blighted shoot tips of eastern hemlock were observed at widely separated locations in the Chattahoochee National Forest in north Georgia. Damage did not appear to be directly related to hemlock woolly adelgid (*Adelges tsugae*) activity, which was sporadic or absent in some areas where symptoms were observed. A preliminary survey in March 2010 revealed that incidence of blighted shoots on individual trees varied, but was as high as 70%. Stems of shoots produced the previous year were frequently necrotic, had lost needles, and bore pycnidia with hyaline, two-celled conidia consistent with those of *Sirococcus tsugae*. Later in the spring and summer, shoots of the current year's growth became blighted, with sporulation of *S. tsugae* also on dead and dying needles. While *S. tsugae* previously has been reported on *T. heterophylla*, *T. mertensiana*, *Cedrus atlantica*, and *C. deodara* in western North America, it has only recently been reported on eastern hemlock, and its ability to induce shoot blight had not been proven. Pure cultures were obtained on streptomycin-amended potato dextrose agar (PDA) and their identity was confirmed by species-specific PCR primers. Nuclear rDNA internal transcribed spacer sequence also was obtained and was identical to sequences for *S. tsugae* previously deposited in GenBank. Two isolates were used to inoculate potted 2-year-old eastern hemlock seedlings in a growth chamber at 20°C with a 16-h photoperiod. Conidia were collected by flooding 1-month-old colonies on PDA with sterile water. Expanding shoots on one branch of each seedling were wounded using scissors to cut the tips off needles and stems, while another branch remained unwounded. Ten seedlings per isolate were inoculated by spraying to runoff with a conidial suspension sterile water, and five similarly treated control seedlings were sprayed with sterile water. Seedlings were covered with plastic bags to maintain high humidity for 4 days. Symptoms were evaluated and reisolation was attempted on streptomycin-amended PDA 2 months after inoculation. Symptoms of seedlings inoculated with either isolate included chlorotic and necrotic needle spots, browning of cut edges of needles, browning and death of needle tips and entire needles, death of stem tips with retention of dead needles, and needle loss. Symptoms of control seedlings were limited to slight browning of cut edges of needles. The fungus was reisolated from wounded shoots of 17 of 20 inoculated seedlings and nonwounded shoots of 5 of 20 inoculated seedlings and was not cultured from control seedlings. To our knowledge, this is the first report of *S. tsugae* in Georgia and also the first demonstration of its ability to produce symptoms that have been attributed to it on any tree species.

Diseases on Horse Chestnut in Norway

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Horse chestnut (*Aesculus hippocastanum*) is mainly found in gardens, public parks and alleys in Norway. It has been known as a healthy tree, but since 2006, a number of new diseases have occurred, likely entering the country by imported horse chestnut transplants.

Leaf blotch (*Guignardia aesculi*) was found in south eastern Norway in August 2006.

Diseased leaves had brown, irregular blotches with a yellow halo around, and heavy infected leaves were wrinkled. The disease became widespread in Europe after it was first detected in 1950. Probably the North Sea, which acts a barrier for natural spread of diseases into most areas of Southern Norway, stopped the pathogen from spreading to the north.

Powdery mildew (*Uncinula flexuosa*) was also discovered in August 2006, while searching for trees with leaf blotch symptoms. Infected leaves had a gray appearance, but the disease was not widespread at the time. By 2010, horse chestnut in several locations in south eastern Norway had heavy powdery mildew attacks. The first detection in Europe was in Germany in 1999.

Anthracnose (*Colletotrichum acutatum*) was found in 2006 on horse chestnut leaves, along the midrib and veins. In one case, *C. gloeosporoides* was detected on horse chestnut leaves from western Norway. ITS sequencing was used to distinguish the two *Colletotrichum* species. It is known from USA that anthracnose may lead to early leaf drop.

Bleeding canker (*Pseudomonas syringae* pv. *aesculi*) is the latest and most serious of the recently discovered pathogens on horse chestnut in Norway. It was detected in south western Norway in June 2010. Diseased trees showed dieback symptoms in the crown and had bleeding canker wounds in the bark. In Europe, the bacterium was first detected in the Netherlands in 2002, and has since been found in several European countries.

Although the horse chestnut leaf miner (*Cameraria ohridella*) has not yet found its way to Norway, the diseases mentioned here may reduce the esthetical value of horse chestnut to such an extent that we no longer recommend it for planting in Norway.

Dothistroma septosporum and *Lecanosticta acicola* in the Czech Republic: Current Situation and Inoculation Tests

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Severe spreading of Needle Blights has been noticed within past 15 years. Ascomycetes *Dothistroma septosporum* (Dorog.) Morelet (teleomorph: *Mycosphaerella pini* Rostr.) and *Dothistroma pini* Hulbary (teleomorph: unknown) causing Dothistroma Needle Blight (Red Band Needle Blight) and *Lecanosticta acicola* (Thüm.) Syd. (teleomorph: *Mycosphaerella dearnessii* M. E. Barr), causing Brown Spot Needle Blight, are considered as the causal agents. The occurrence of these pathogens from new sites as well as from new host tree species has been reported. Scandinavia can be an example of such crucial spreading of the disease. The Red Band Needle Blight has been occurring in northern Europe since the year 2008 in a big scale mainly on *Pinus sylvestris*. Woods et al. (2005) consider DNB as a disease supported by global climate change. He emphasizes the influence of locally enhanced precipitations to the spreading of the disease in temperate zone. The increasing amount of new infested sites has been noticed also in the Czech Republic. The fast disease spreading plays the key role in this current situation. The observation of the disease development has been carrying out in 132 sites with host tree species so far. DNB has been confirmed on 79 sites and from 23 of those pure cultures have been successfully cultivated. The occurrence of Brown Spot Needle Blight is not too alarming in the CZ as DNB from the point of the amount of infested sites. Nevertheless Brown Spot Needle Blight in Southern Bohemia occurs in a big scale hence its spreading to new areas is not out of the question.

The current occurrence of Needle Blights in a huge scope and its relatively easy and fast spreading has became the main reason of the inoculation spraying test. The purpose of the inoculation test is to verify the symptomology of Needle Blights. The inoculation test will be done simultaneously with the Real-Time PCR, whereby only a little DNA concentration of the organism in a sample can be detected. By this it is possible to detect an occurrence of a disease in its early stage, which is a problem for diagnostics. The identification of the pathogen in the early stage could help the use of protective spray for prevention of the occurrence and spreading the disease. Another purpose of the inoculation test is the question of susceptibility of various conifer species on the *Dothistroma* and *Lecanosticta* infection. Inoculation tests have been carrying out since the spring 2010 and a quarantine greenhouse in Praha-Ruzyne and former Hacker's nursery have been chosen for the purpose of this experiment. Pure cultures of *D. septosporum* and *L. acicola* were used as a basic inoculation suspension, whereas their conidias were washed by double distilled water. Inoculation suspension was applied on seedlings with atomizer. Selected seedlings were consequently covered with unwoven fabric for encouraging favourable microclimatic conditions. Following seedling species have been placed in the greenhouse or/and planted *in natura* in former nursery: *Pinus nigra, P.mugo, P.sylvestris, P.uncinata, Picea pungens, P.abies, P.sitchensis a Pseudotsuga menziesii*.

With respect to actual epidemic situation in many countries, it is necessary to discuss the role of climatic factors in Europe and trade with plant material as the main risk factor for the spreading of both diseases.

Control of Chestnut Canker with Hypovirulent Strains of *Cryphonectria parasitica* in Castilla y León (Spain)

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Hypovirulence, in with a virus reduce the virulence of the fungus, is a biological control for *Cryphonectria parasticia* used with effectiveness in different European countries since his first detection in Italy. In this study, the objective was to determine if the hypovirulent isolates found in the field can be used for controlling the expansion of chestnut canker in Castilla y León. During 2007 and 2008 four inoculation assays were conducted in chestnut stands in Castilla y León. Three of the inoculations were done in León were the hypovirulence has been found naturally distributed in different orchards. One assay was done in Zamora were no hypovirulence has been found so far. The first inoculation was conducted in autumn 2007 with hypovirulent isolates of the vegetative compatibility group EU11. In 2008 the inoculations were conducted in spring and autumn with isolates from EU11 and EU1. At the inoculation time the cankers were measured and the area was calculated with the ellipse formula. The effectiveness of the inoculation was measured calculating the relative increment of growth after 6, 12 or 18 months since inoculation. In autumn the inoculations, had good results reducing the growth of the cankers with all the treatments assayed after 12 or 18 months. Both vcg tested, EU1 and EU11 reduced the canker growth. The inoculation conducted in spring had no differences between the inoculated and the control cankers in exception of one treatment in Zamora. All the isolates used were efficient controlling canker growth and the best moment to conduct the inoculation was autumn. The current vcg distribution and the number of hypovirulent strains isolated represent a good opportunity for an effective biological control of chestnut canker in Castilla y León.

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