Welcome to the

Joint IUFRO Working Party Meetings

7-12 June, 2015 in Uppsala, Sweden

7.02.02 - Foliage, shoot and stem diseases of forest trees &7.03.04 - Diseases and insects in forest nurseries



Hosted by the Swedish University of Agricultural Sciences

Program Monday 8 June

08:00-08:30	Late registration, hanging of posters
08:30–09:00	Welcome by Johan Schnürer (Pro Vice-Chancellor of SLU) (15 min), general information of the meeting (15 min + 5 min questions)
09:00-09:30	Keynote speaker: Marco Pautasso (EFSA, Italy & ETH, Zurich): "Forest health in a changing world" (25 min + 5 min questions)
09:30-10:00	Coffee break
10:00-12:00	EMERGING DISEASES CAUSED BY DOTHISTROMA AND DIPLODIA (session leader Asko Lehtijärvi)
10:00-10:15	<u>Stanosz, G.R.</u> , Smith, D.R. Albers, J. The shoot blight and canker pathogen <i>Diplodia scrobiculata</i> and asymptomatic seedlings in natural stands of <i>Pinus banksiana</i>
10:15-10:30	Doğmuş-Lehtijarvi, H.T., Yeltekin, Ş. Aday Kaya, A.G. Lehtijarvi, A. Disease severity of <i>Diplodia sapinea</i> on some pine plantations
10:30-10:45	Fraser, S., Brown, A. <u>Woodward, S.</u> Variation in sceptibility of Scots and Lodgepole pine provenances to infection by <i>Dothistroma septosporum</i>
10:45-11:00	<u>Millberg, H</u> ., Hopkins, A. Boberg, J. Davydenko, K. Stenlid, J. Development of Dothistroma needle blight on seedlings of <i>P. sylvestris</i> and <i>P. contorta</i> in central Sweden
11:00-11:15	<u>Lehtijärvi, A</u> ., Doğmuş Lehtijärvi, H.T. Oskay, F. Woodward, S. Dothistroma septosporum in Turkey
11:30-12:00	Session discussion and wrap up
12:00-13:00	Lunch at Syltan canteen
13:00-15:00	DETECTION OF NATIVE AND INVASIVE SPECIES IN NURSERIES (Session leader: Scott Enebak)
13:00–13:15	<u>Alonso Chavez, V</u> ., van den Bosch, F., Parnell, S. Strategies for monitoring of invasive tree epidemics in tree nurseries for disease detection and tracking
13:15–13:30	<u>Aday Kaya, A.G</u> ., Karakaya, A. Doğmuş-Lehtijarvi, T. Lehtijärvi, A. Oskay, F. Diseases of coniferous and deciduous seedlings in forest nurseries in Marmara Region in Turkey
13:30–13:45	Weiland, J.E., Beck, B.R. Davis, A. Grunwald, N.J. Santamaria, L. Littke, W.R. Browning, J.E. Edmonds, R.L Understanding <i>Pythium</i> species in forest nurseries of the Northwest United State

13:45-14:00	Leon, A.L., Chastagner, G.A. The utility and limitations of molecular diagnostic technologies in forest seedling nurseries	
14:00-14:15	Session discussion and wrap up	
14:15-14:45	Coffee break	
	CANKER DISEASES (session leader Isabel A. Munck)	
14:45-15:00	Luchi, N., Capretti P., Migliorini D., Santini A. Caliciopsis canker: a damaging disease on pine plantations in Italy	
15:00–15:15	<u>Munck, I.A.</u> , Broders, K. William Livingston, W. Lombard, K. Luther, T. Ostrofsky, W. Extent and severity of Caliciopsis canker in New England, U.S.A: an emerging disease of eastern white pine	
15:15–15:30	<u>Samils, B</u> ., B. Barklund, P. Kaitera, J. Stenlid, J. Population structure of <i>Cronartium flaccidum</i> and <i>Peridermium pini</i> in relation to the current epidemic	
15:30–15:45	<u>Chastagner, G.A.</u> , Riley, K. Coats, K. Talgø, V. Grovesiella and Neonectria canker – two diseases of <i>Abies</i> in the Pacific Northwest United States	
15:45–16:00	<u>Dunnell, K.L.</u> , LeBoldus, J.M. Using <i>Populus nigra</i> to improve hybrid poplar resistance to Septoria canker in the United States	
16:00–16:15	<u>Prospero S</u> ., Rigling D. Using molecular tools to assess establishment and spread of the biological control agent of chestnut blight	
16:30-17:00	Session discussion and wrap up	
17:00-17:30	Leg strecher	
17:00-17:30	IUFRO presentation by Julio J. Díez (coordinator of the WP 7.02.02)	
18:00-18:15	Practical information	
18:15-19:00	Poster session	

Program Tuesday 9 June

09:00-09:30	Keynote speaker: Richard Hamelin (UBC, Canada): "The making of a tree canker pathogen" (25 min + 5 min questions)
09:30-10:00	Coffee break
10:00-12:00	ASH DIEBACK (Session leader Michelle Cleary)
10:00-10:15	Stenlid, J., Cleary, M. Brandström-Durling, M. Elfstrand, M. What makes the ash dieback fungus <i>Hymenoscyphus fraxineus</i> pathogenic?
10:15-10:30	<u>Hrabětová, M</u> ., Havrdová, L. Černý, K. Evaluation of fungicides for control of ash dieback in forest nurseries
10:30-10:45	<u>Dvořák, M</u> ., Rotková, G. Spore dispersal of <i>Hymenoscyphus fraxineus</i> in the Czech Republic
10:45–11:00	<u>Nguyen, D</u> ., Cleary, M.R. Enderle, R. Berlin, A. Stenlid, J. Local spatial population dynamics in the Ash dieback pathogen <i>Hymenoscyphus fraxineus</i>
11:00-11:15	Botella, L. Čermáková, V. Rozsypálek, J. Bačová, A. Jankovský, L. In vivo and in vitro characterization of the pathogenicity of Hymenoscyphus fraxineus: research in perspective of ongoing COST FP1103 FRAXBACK Action
11:15–11:30	<u>Cleary, M</u> ., Nguyen, D. Marčiulynienė, D. Berlin, A. Vasaitis, R. Stenlid, J. <i>Hymenoscyphus fraxineus</i> on <i>Fraxinus mandshurica</i> in Far East Russia: genetic diversity, associated fungal community and evidence of endophytic lifestage
11:30-12:00	Session discussion and wrap up
12:00-13:00	Lunch at Syltan canteen
13:00-15:00	GREMMENIELLA (Session leader Leticia Botella)
13:00-13:15	<u>Capretti, P., Migliorini, D. Botella, L. Luchi, N.</u> Northward migration of <i>Gremmeniella abietina</i> from Italian peninsula
13:15–13:30	Laflamme, G., Rioux, D. Two-year survival of <i>Gremmeniella abietina</i> conidia collected on branches left on the ground after pine harvesting
13:30–13:45	<u>Romeralo, C</u> ., Witzell, J. Romeralo-Tapia, R. Botella, L. Diez, J.J. Antagonistic activity of fungal endophyte filtrates against <i>Gremmeniella</i> <i>abietina</i> infections on aleppo pine seedlings

13:45–14:00	<u>Botella, L</u> ., Dvořák, M. Vainio, E.J. Jankovsky, L. Capretti, P. Hantula, J. Diez, J.J. Luchi, N. Interactions among temperature, a novel mycovirus and the conifer pathogen <i>Gremmeniella abietina</i>	
14:00-14:15	Session discussion and wrap up	
14:15-14:45	Coffee break	
14:45–16:30	FOLIAR DISEASES ON CONIFER TREES (Session leader Gabriella Ritóková)	
14:45-15:00	<u>Jalkanen, R.</u> , Notes on needle pathogens on larch in northern Finland	
15:00–15:15	Tunalı, Z., <u>Oskay, F</u> . Lehtijärvi, A. Aday Kaya, A.G. Doğmuş Lehtijärvi, H.T. In planta detection of needle pathogens in <i>Pinus brutia</i> needles	
15:15–15:30	<u>Aday Kaya, A.G</u> ., Lehtijärvi, A. Tunalı, Z. Yeltekin, Ş. Doğmuş-Lehtijärvi, T. Fungal endophyte communities in needles differ between endemic and exotic pine species in nearby plantations in Turkey	
15:30–15:45	<u>Adamson, K.</u> , Drenkhan, R. Puidet, B. Kalder, R. Hanso M. Invasive pathogens on pines (<i>Pinus</i> spp.) in Northern Baltics	
15:45–16:00	<u>Pérez-Sierra, A</u> ., Hendry, S. Gorton, C. Lewis, A. Kalantarzadeh, M. Sancisi-Frey, S. Brown, A. Cedars under threat from Sirococcus spp.	
16:00–16:15	<u>Talgø, V.</u> , Skage, J.O. Steffenrem, A. Johnskås, R. Damage by <i>Delphinella abietis</i> on subalpine fir in Norway may depend on the origin of the seed source	
16:15–16:30	<u>Ritóková, G</u> ., Shaw, D. Swiss needle cast foliage disease of Douglas-fir (<i>Pseudotsuga menziesii</i>) in the U.S. Pacific Northwest	
16:30–16:45	<u>Bennett, P.I.</u> , Stone, J.K. Reproductive isolation and population structure in the Douglas-fir Swiss Needle Cast (SNC) pathogen, <i>Phaeocryptopus gaeumannii</i> , in the U.S. Pacific Northwest	
16:45–17:15	Session discussion and wrap up	
17:15-17:30	Practical information	
17:30-18:45	Poster session	

Program Wednesday 10 June

09:00–09:30	Keynote speaker: Jason Smith (UF, US): "Laurel wilt – a Trojan horse inside an ambrosia beetle" (25 min + 5 min questions)	
09:30-10:00	Coffee break	
10:00-12:00	FOLIAR DISEASES IN BROADLEAVED TREES (Session leader M.L. Desprez-Loustau)	
10:00–10:15	<u>Oskay, F.,</u> Doğmuş Lehtijarvi, H.T. Lehtijarvi, A. Impact of boxwood blight pathogens; <i>Cylindrocladium buxicola</i> and <i>Pseudonectria buxi</i> on <i>Buxus balearica</i> and <i>Buxus sempervirens</i>	
10:15–10:30	<u>Vasaitis, R</u> ., Menkis, A. Östbrant, I-L. Wågström, K. Dutch elm disease in Baltic sea island of Gotland	
10:30–10:45	<u>Smith, D. R.</u> , Stanosz, G. R. Preliminary analyses of ITS sequence variation among Marssonina leaf spot and shoot disease pathogens of poplars	
10:45–11:00	<u>Desprez-Loustau, M.L</u> ., Dezette, D. Fabreguettes, O. Massot, M. Piou, D. Marçais, B. Spatial distribution of sibling species causing European oak powdery mildew	
11:15–11:45	Session discussion and wrap up	
11:45-12:00	Practical information on field trip	
12:00-13:00	Lunch at Syltan canteen	
13:00-15:00	PINE PITCH CANKER (Session leader Jorge Martín-García)	
13:00–13:15	<u>Martínez-Álvarez</u> , P., Fernández-González, R.A. Sanz-Ros, A.V. Pando, V. Diez, J.J. Evaluation of the antagonism produced by fungal endophytes against <i>Fusarium circinatum</i> in the field	
13:15–13:30	<u>Bezos, D</u> ., Martínez-Álvarez, P. Diez, J.J. Fernández, M.M. <i>Pityophthorus pubescens</i> associated with <i>Fusarium circinatum</i> in northern Spain	
13:30–13:45	Elvira-Recuenco, M., Iturritxa, E. Raposo R. Impact of seed transmission on the infection and development of pitch canker disease	
13:45–14:00	<u>Martín-García, J</u> ., Woodward, S. Vannini, A. Vettraino, A.M. Alves, A. Hantula, J. Bragança, H. Capretti, P. Diez, J.J. COST Action FP1406: Pine pitch canker - strategies for management of Gibberella circinata in greenhouses and forests (PINESTRENGTH)	
14:00-14:15	Session discussion and wrap up	

Coffee break	
DECLINE DISEASES (Session leader Sarah Green)	
<u>Prieto-Recio, C</u> ., Martín-García, J. Bravo, F. Diez, J.J. Abiotic factors involved in <i>Pinus pinaster</i> decline in the Iberian Peninsula	
Doonan, J., Denman, S. Pachebat, J.A. Golyshin, P.N. McDonald, J.E. Genomic analysis of bacteria associated with Acute Oak Decline	
<u>Brown, N</u> ., Parnell, S. Denman, S. Filipe, J. Jeger, M. and Bosch, F. Monitoring forest diseases and syndromes: the case of Acute Oak Decline	
<u>Sapp, M</u> ., Lewis, E. Thwaites, R. Kirk, S. Denman, S. Elphinstone, J. Identification of bacteria associated with the acute oak decline syndrome in England using Next Generation Sequencing	
<u>Denman, S</u> ., Plummer, S. Sumner, M. Reed, K. Peace, A. Susan, K. Simulating key elements of Acute Oak Decline to produce signs and symptoms in log tests	
Nowell, R.W., <u>Green, S</u> ., Laue, B.L, Sharp, P.M. Evolutionary adaptations of Pseudomonas syringae pv. aesculi to the European horse chestnut	
Session discussion and wrap up	
Leg stretcher	
MISCELANIOUS DISEASES (Session leader Johanna Boberg)	
Zamora, C, Haque, H., Diez, J.J, <u>Martín-García, J</u> . Inoculation tests of <i>Phytophthora alni</i> and <i>P. plurivora on</i> alder seedlings	
Muñoz-Adalia, E.J. Fernández, M.M. Romeralo, C. Diez, J.J. The use of mycoviruses in forest disease management	
<u>Sturrock, R.N.</u> Decay of living western redcedar: overview of an information synthesis and new research in progress	
<u>Fraser, S</u> ., Brown A. Woodward, S. Inter- and intra-specific variation in susceptibility to dothistroma needle blight in Britain. How susceptible are <i>Pinus sylvestris</i> and <i>Pinus contorta</i> ?	
<u>Bellahirech, A</u> ., Inácio, M.L. Nóbrega, F. Bonifácio, L. Sousa, E. Ben Jamâa, M.L. New fungal associates of the wood boring beetle <i>Platypus cylindrus</i> F. (Coleoptera: Curculionidae) with special focus on <i>Raffaelea montetyi</i> fungi in Tunisian cork oak stands	
Session discussion and wrap up	

Program Thursday 11 June

FIELD TRIP AND GALA DINNER

08:00	Meeting in Uppsala Konsert & Kongress	
08:15–9:00	Trip to ash stand to look at <i>H. fraxineus</i> damages.	
09:00–9:45	Ash dieback in Sweden (15 min). Presented by Jan Stenlid . Participants are divided in two groups i.e. two buses A and B.	
09:45-10:00	Trip to Lugnet plantskolan.	
10:00-12:00	<u>Bus A</u> makes a tour in Nursery (1 hour). Person responsible: Audrius Menkis . <u>Bus B</u> , Norway spruce plantation to validate resistance markers for <i>Heterobasidion parviporum</i> (1 hour). Presented by Jan Stenlid .	
11:00-12:00	Bus A visits Norway spruce plantation, and Bus B visits nursery.	
12:00-12:45	Lunch in Lugnet plantskolan.	
13:00-14:30	Trip to Riddarhyttan.	
14:30-15:30	Visit <i>Gremmeniella</i> attacked pine forest Presentations by Jan Stenlid and Ulf Sikström .	
15:30-16:00	Trip to Fagersta	
16:00-17:00	Visit to <i>Dothistroma</i> attacked site. Presentation by Hanna Millberg . Coffee and small snack will be served.	
17:00-19:00	Trip back to Uppsala.	
20:00	Gala dinner in Norrlands Nation (Västra Ågatan, 753 09 Uppsala 018-65 70 70).	
20:30	Dinner is served.	
22:30-00:15	Dancing with rock band "The electric rubber tub".	
00:15-01:00	Pub	

Program Friday 12 June

09:00-10:10	NURSERY PEST AND DISEASES (Session leader: Tugba Doğmuş-Lehtijarvi)
09:00–09:15	<u>Enebak, S.A.</u> , Starkey, T.E. Soil fumigation techniques used in the production of forest-tree seedlings in the southern United States
09:15–09:30	<u>Stenström, E.</u> , Gadjieva, R. Ihrmark, K. Stenlid, J. First report of Rosellinia spp. on seedling of <i>Pinus sylvestris</i> and <i>Picea abies</i> during cold storage in a forest nursery in Sweden
09:30-09:45	<u>Menkis, A</u> ., Stenlid, J. Stenström, E. Rhizosphere fungi of nursery grown conifer seedlings
09:45–10:00	<u>Hezil, S</u> ., Chakali, G. The strategic occupation of processionary moth <i>Thaumetopoea pityocampa</i> in the semi-arid Aleppo pine forests (Algeria)
10:00-10:30	Session discussion and wrap up
10:30-11:45	Business meeting
11:45-12:00	Closing conference and departure

Organising committee

DR. JONÀS OLIVA, DR. AUDRIUS MENKIS AND PROF. JAN STENLID Dept. Forest Mycology and Plant Pathology, SLU (Sweden).

Scientific committee

JONAS OLIVA	Dept. Forest Mycology and Plant Pathology, SLU (Sweden).
JAN STENLID	Dept. Forest Mycology and Plant Pathology, SLU (Sweden).
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KATHY J. LEWIS	University of Northern British Columbia (Canada).
LIBOR JANKOVKSY	Mendel University (Czech Republic).

Oral presentations

Monday 8 June

EMERGING DISEASES CAUSED BY DOTHISTROMA AND DIPLODIA

DETECTION OF NATIVE AND INVASIVE SPECIES IN NURSERIES

CANKER DISEASES

The shoot blight and canker pathogen *Diplodia scrobiculata* and asymptomatic seedlings in natural stands of *Pinus banksiana*

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Frequent and severe damage caused by the Diplodia shoot blight and canker pathogens of conifers has affected red pines (P. resinosa) and jack pines (P. banksiana) in the northcentral USA. Seed cones on which D. sapinea and D. scrobiculata sporulate are sources of abundant inoculum. Red pines may acquire D. pinea but initially remain asymptomatic, with later pathogen proliferation and disease development under conditions that induce host stress. Relatively less is known about relationships of these pathogens with jack pines, although D. scrobiculata has been more frequently associated with jack pines than red pines. The potential for asymptomatic persistence of *D. sapinea* or *D. scrobic*ulata on or in asymptomatic seedlings in understories of natural jack pine stands was investigated in Minnesota, USA. Young seedlings were collected in four locations in fall 2013 and four locations in fall 2014. A lower stem/root collar segment from each seedling was surface-disinfested, incubated on tannic acid medium, and later examined for pycnidia with conidia characteristic of these species. Approximately 20% and 35% of seedlings collected in 2013 and 2014, respectively, were culturally positive. Additionally, species-specific primers were used to determine which of these two Diplodia species were cultured from seedlings collected in 2014. Both pathogens were detected, but the overwhelming majority of tests for which results were obtained were positive for D. scrobiculata. Early acquisition of Diplodia pine shoot blight and canker pathogens by the next generation of jack pines maintains these pathogens at infested sites, with potential for disease development during conducive conditions.

Disease Severity of Diplodia sapinea on Some Pine Plantations

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Diplodia sapinea (Desmaz.) J. Kickx [syn. *Sphaeropsis sapinea* (Fr.: Fr.) Dyko & Sutton] causes different types of diseases on conifers: stunting of new growth, browning of needles, shoot blight, twig and branch dieback, crown wilt, bark cankers, root disease, damping- off, root rot, and as saprophyte blue stain of sapwood of fallen or freshly cut timber. The fungus has worldwide distribution in the temperate and tropical regions. Among different hosts *Pinus* species (especially *Pinus nigra* Arnold, *P. radiata* Don. and *P. sylvestris* L.) are the most susceptible ones.

In this study we investigated the disease severity caused by *D. sapinea* on *P. nigra*, *P. sylvestris*, *P. taeda*, *P. pinaster*, *P. radiata* and *Pseudotsuga menziesii* plantations. Disease surveys were carried out in May 2012 in İzmit in the Marmara region of Turkey. The plantations were investigated using crosswise sampling strategy. Trees were sampled along transects, in which every fifth tree was investigated for the presence of *D. sapinea*. For each sampled tree the disease severity was scored.

D. sapinea was found to be the causal agent of the dieback in the surveyed plantations. There were differences in disease severity among the stands. The *P. nigra* and *P. sylves-tris* stands had the highest average whole crown disease severity. In the *P. radiata* and *P. pinaster* stands, the average disease severity in the upper and the lower part of the crown did not differ significantly between the stands.

Variation in susceptibility of Scots and Lodgepole pine provenances to infection by Dothistroma septosporum

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Both artificial inoculations and field infections were used to determine variations in susceptibility of six different Scottish sources of native *Pinus sylvestris* to infection and damage by the needle blight pathogen *Dothistroma septosporum*. The inoculation protocol was modified from a system developed in New Zealand in order to infect larger numbers of young pines. Field infections were monitored in young trees planted out at two locations, Culbin in north-east Scotland and Torrs Warren in south-west Scotland. In artificial inoculations, plants sourced from Glen Carrich and Glen Loyne showed the highest levels of symptoms, compared to low levels of infection in plants from Amat, Ben Eighe and Abernethy. Significant differences in disease levels between sources were observed at both Culbin and Torrs Warren, although overall, much more disease occurred at Torrs Warren than at Culbin. At Culbin, trees from Benn Eighe showed the most infection, although levels of infection in all plants were low compared with Torrs Warren. At Torrs Warren, plants sourced from Glen Carrich, Glen Loyne and Abernethy had the highest levels of infection. The possibility of using artificial inoculations to test relative susceptibility of pines to infection by *D. septosporum* is discussed.

Development of Dothistroma needle blight on seedlings of *P. sylvestris* and *P. contorta* in central Sweden

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D. septosporum was identified for the fist time in Sweden in 2007. To learn more about Dothistroma needle blight under Swedish conditions, the disease development was followed on seedlings of *Pinus sylvestris* and *Pinus contorta* var. *latifolia*, planted in two forest sites in central Sweden. Needle samples were collected every second to third month for a two-year period, and the seedlings were also examined for typical red band and fruit bodies. Infections of *D. septosporum* were detected in needles samples using PCR with species specific. Young needles of both host species became infected already during the first summer, as they were starting to elongate and the first conidiomata appeared the following spring. On older needles of *P. sylvestris*, new fruit bodies developed in the autumn the same year as the needles were infected. Many seedlings carried latent infections without any symptom development. There was no clear difference in the susceptibility of the two host species, nonetheless, at the same site, the mortality of *P. sylvestris* was higher than *P. contorta*. However, stress and the presence of *L. seditiosum* infecting *P. sylvestris*.

Dothistroma septosporum in Turkey

Lehtijärvi, A¹. Doğmuş Lehtijärvi, H.T.² Oskay, F.³ and Woodward, S.⁴

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Dothistroma needle blight (DNB), is a serious disease of conifers, particularly pines, caused by the fungi *Dothistroma septosporum* and *Dothistroma pini*. Although reports of the occurrence of DNB in Turkey go back to 2001, little is known about the disease including its distribution, hosts, and the Dothistroma species present in Turkey, population diversity and virulence of the pathogen. A major outbreak of DNB occurred in 2013, however, in south western Turkey, on the most widely distributed pine species (*Pinus brutia* Ten.), drawing much greater attention to this highly damaging forest pathogen in Turkey.

To date, only *D. septosporum* has been found in the current outbreak area in south western Turkey, focused on *P. brutia*. Moreover, presence of *D. septosporum* was also confirmed on seedlings of *P. nigra* from a state forest nursery in Thrace, North-West Turkey. Preliminary studies with species-specific mating type primers showed that both mating types of *D. septosporum* were present on the same trees and in the same area. Moreover, characterisation of 12 *D. septosporum* isolates from *P. brutia* growing in the same region indicated a high level of genotypic diversity, even in a small sample size, which yielded 8 multilocus haplotypes. The possible significance of these results in terms of the future of pine management in Turkish forests is discussed.

Strategies for monitoring of invasive tree epidemics in tree nurseries for disease detection and tracking

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The movement of trees and plant products across the globe has increased in recent years, triggering an increase of introduced plant pathogens. Tree nurseries importing material from abroad may play a role in the introduction and spread of tree diseases. A recent example suggests that ash dieback could have been introduced into the UK through nursery trade, as well as through aerial spread. The economic, environmental and social costs associated with the spread of invasive pathogens become considerably larger as the incidence of the pathogen increases. To control the movement of pathogens across the plant trade network it is crucial to develop efficient sampling and monitoring programmes at key points of the network. By detecting the introduction of invasive pathogens at low incidence, the control and eradication of an epidemic is more likely to be successful.

Here, we develop a simple epidemiological model to detect and trace invasive plant pathogens in a tree nursery. Using statistical methods, we predict the epidemic incidence given that a detection of the pathogen has occurred for the first time [1]. Equally, we trace forward the probability of pathogen movement into other parts of the trade-network. We explore the effect of these results on data for Ash dieback in the UK. These results provide new insight for the design of monitoring strategies at key points of the trade network.

[1] Parnell, S., et al. J, Ttheor. Biol. 305 (2012): 30-36

Diseases of coniferous and deciduous seedlings in forest nurseries in marmara region in Turkey

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The 122 state forest nurseries located in 62 cities all around Turkey have a capacity of 496 million seedlings per year. The current annual production is 450 million. Success of afforestation depends on the usage of healthy seedlings. The presence of *Phytophthora* and some fungal species in nurseries is an emerging problem for afforestation.

Over the last two years we have intensively surveyed local nursery fields for presence of diseases caused by *Phytophthora* and some fungal species. In total, 9 important forest nurseries in Thrace in north-western Turkey were surveyed. One hundred twenty potted seedlings showing symptoms were sampled. Roots, stem and the foliage part of the seedlings were investigated under stereomicroscope for the presence of structures of disease causing agents. Isolations from symptomatic roots were performed on selective PDA medium. Soils of the seedlings were baited with young leaves of carnation, rhodo-dendron and cork oak. Infected leaves were placed into petri dishes containing selective PARPNH agar. Morphological identification was based on colony patterns, growth rates, and morphological features. Ribosomal DNA internal transcribed spacer sequences of 820 bp long products were amplified using ITS1 and ITS4 primer pair.

Abies bornmülleriana, Quercus virginiana, Thuja occidentalis, Pinus sylvestris, Quercus suber, Platanus orientalis, and Buxus sempervirens seedlings were found to be infected by Pythiaceous species and root-rot fungi. Three different Phytophthora, two different Phytopythium, Fusarium oxysporum, F. moniliforme and Cylindrocarpon destructans were identified by morphological and ITS region sequence comparisons. We also found Dothistroma septosporum on Pinus brutia seedlings.

Understanding Pythium species in forest nurseries of the Northwest United States

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Forest nurseries in the Northwest United States produce 250 million tree seedlings (70% Douglas-fir, Pseudotsuga menziesii) for reforestation each year. However, production is severely constrained by several soilborne plant pathogenic genera, including Pythium. Soil fumigation with methyl bromide is still regionally common for controlling soilborne pathogens. But, as fumigation becomes progressively more regulated, information about the identity, biology, and epidemiology of Pythium species will be increasingly important to develop new, effective disease control measures. Pythium species diversity was evaluated at three forest nurseries and was found to differ by nursery: each nursery had a unique Pythium community consisting of 10-14 species. Furthermore, each species differed in its ability to cause damping-off of Douglas-fir, with seedling mortality varying from 0 to 54% depending on the species. Subsequent in vitro fungicide and biological control sensitivity studies found differences in *Pythium* species responses to mefenoxam and Streptomyces lydicus, but not to fosetyl-Al. Mefenoxam resistance was found in P. ultimum at two of the nurseries. Finally, population genetic analyses of P. irregulare, P. sylvaticum, and P. ultimum revealed that Pythium isolates, including fungicide-resistant isolates, have been moved among forest nurseries. These results illustrate the potential difficulties that might be encountered when trying to manage diseases caused by communities of soilborne plant pathogens.

The utility and limitations of molecular diagnostic technologies in forest seedling nurseries

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Disease diagnostics in forest pathology are constantly improving as new molecular technologies are discovered and older technologies become more accessible and more affordable. Older techniques like PCR and sequencing allow for identification of a single organism which is useful when studying new and emerging pathogens. Newer technologies, such as metagenomics, allow us to understand the composition of soil microbiomes. The use of these and other molecular technologies show promise in forest nurseries by providing accurate diagnostic information on the pathogens that impact seedling growth and development. However, there are also drawbacks and limitations in the implementation of these procedures. A quantitative real-time PCR (qPCR) assay was developed to quantify Fusarium commune and distinguish it from the less virulent species, F. oxysporum, in bareroot Douglas-fir (Pseudotsuga menziesii) seedling nurseries. Testing of the qPCR assay was successful in controlled greenhouse experiments and in addition to quantifying F. commune, the assay will be useful for examining its pathogenicity, life cycle, behavior and control. However, the assay faces challenges in the field from soil inhibitors, scale of sampling, and cost. Each nursery and crop will likely benefit from different technologies and have its own suite of limitations to contend with, but even so, current advances in molecular diagnostics offer great promise to help detect nursery diseases.

Caliciopsis canker: a damaging disease on pine plantations in Italy

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Caliciopsis canker is a fungal disease of conifers in Europe and North America. During the last two years a *Caliciopsis pinea* outbreak has been observed in some plantations of *Pinus radiata* in central Italy, occasionally the fungus was found on *P. pinaster*. *C. pinea* is considered a weak pathogen that usually affects trees with low vigour commonly growing in unthinned stands. Symptoms of infections include cankers on stems and shoots with resin bleeding. In Europe the disease has been described in the past years on *P. radiata* and several different native pine species, such as *P. pinea*, *P. pinaster* and *P. halepensis*. To assess the impact of Caliciopsis on native and exotic pine hosts two different experiments were carried out: a) fungal growth of different *Caliciopsis pinea* strains by using agarized media containing ground needle pine (pine needle agar, PNA); b) pathogenicity test on young pine seedlings of susceptible species.

Results showed that Caliciopsis was able to grow on PNA and to cause significant lesions on challenged seedlings. Our findings confirm that *Caliciopsis pinea* should be considered as a dangerous pathogen of *P. radiata* stands in central Italy, however the outbreaks of disease could be relevant also on native pine species especially if they are subjected to predisposing factors, such as the environmental stresses.

Extent and severity of Caliciopsis canker in New England, U.S.A: an emerging disease of eastern white pine

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Caliciopsis canker is an emerging problem in pine growing regions of eastern North America where *Caliciopsis pinea* infects eastern white pine (*Pinus strobus*), *P. echinata*, and P. virginiana. In Europe, the pathogen infects P. pinaster and P. radiata. Although reports of damage caused by C. pinea were common in the 1930s, since then there has been little progress towards understanding its epidemiology. For example, Caliciopsis canker is associated with overstocked stands and poor soils, but few quantitative data are available. The objective of this study was to identify areas at greatest risk of C. pinea damage. During 2014, 58 sites across New England with >75% white pine basal area were surveyed. Most sites (72%) had Caliciopsis canker signs or symptoms. In sites with Caliciopsis canker, 36% of the overstory pines were symptomatic. Poletimber was more likely to be symptomatic (50% trees) than sawtimber (30% trees) (α =0.05). White pine density for sites with Caliciopsis canker was 311 trees/ha compared to 220 trees/ha for sites without Caliciopsis canker (p=0.1). Caliciopsis canker symptoms occurred more frequently (p=0.1) in sites with excessively drained, coarse textured soils derived from glacial outwash (86%) or sites with poorly drained soils and low fertility (78%) than in sites with well drained, more fertile soils (59%). This is a problem for forest managers because coarse textured soils derived from glacial outwash are common in New England and white pine is the only significant commercial species to grow well on these sites where soil fertility is too poor for hardwoods.

Population structure of *Cronartium flaccidum* and *Peridermium pini* in relation to the current epidemic

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Extensive attacks of Scots pine blister rust are prevailing in northern Sweden, mainly in the counties Norrbotten and Västerbotten. The first reports of damage came in 2002 and an inventory in 2008 showed that 130 000 hectares, or 34% of young pine forests in this region, were infected. A follow-up inventory in 2012 did not indicate any decrease in rust attacks. What induced the sudden epidemic is not known. The blister rust on Scots pine is caused by two forms of the fungus, the macrocyclic host-alternating *Cronartium flaccid-um*, and *Peridermium pini* (syn. *Endocronartium pini*) which spreads clonally from pine to pine. *Melampyrum sylvaticum* is considered to be the most important alternate host of *C. flaccidum* in northern parts of Sweden and Finland, but a number of other plants from various families are also suitable alternate hosts. The two fungi were recently suggested to belong to the same species, since molecular marker data show little overall genetic differentiation between the fungi. It is possible to separate the two forms using molecular markers, since *P. pini* is characterized by totally homozygotic aeciospores, while *C. flaccidum* aeciospores contain heterozygous loci.

In order to study the population genetic structure and geographic distribution of *C. flaccidum* and *P. pini*, a collection of aeciospore samples from 8 different locations in Sweden and 5 locations in Finland was made in 2011. In total 220 samples were analyzed using seven microsatellite markers. Our results showed that *C. flaccidum* dominates the fungal populations in the epidemic areas in Northern Sweden. We could not detect any distinct geographic separation between *C. flaccidum* and *P. pini*, although specific locations or areas were dominated by one or the other form. Populations of *C. flaccidum* in the epidemic areas in Northern Sweden were genetically similar to the neighboring populations in Northern Finland.

Grovesiella and Neonectria canker – Two diseases of *Abies* in the Pacific Northwest United States

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A number of true firs (Abies spp.) are commercially grown as Christmas trees, used in landscaping, used for bough production, grown for timber and/or occur naturally in the Pacific Northwest (PNW) states of Washington, Oregon, and Idaho in the United States. The fungi Grovesiella abieticola and Neonectria neomacrospora cause canker diseases on true firs in the PNW. Neonectria canker is also an emerging problem on true firs in Scandinavia. Symptoms of both diseases typically consist of dead shoots, cankers with heavy resin flow and distal overgrowths, branch flagging and occasionally mortality when cankers completely girdle stems. Grovesiella typically produces characteristic small, gray-black apothecia on the canker's surface, while characteristic red perithecia are occasionally found on cankers produced by Neonectria. Grovesella canker appears to have a more limited host range than Neonectria canker, which was found on 16 hosts in WA and OR during a survey of trees in the PNW in 2013. White fir (A. concolor) and shasta fir (A. magnifica var. shastensis), are very susceptible to Grovesiella canker and support high levels of sporulation. Grovesiella has been observed on seedlings in nurseries and trees in Christmas tree plantations and landscape plantings. N. neomacrospora appears to be limited to trees in Christmas tree plantations and landscape plantings in the PNW. Limited information is available relating to factors that affect the development of these diseases, and management is currently limited to cultural practices, such as sanitation

Using *Populus nigra* to improve hybrid poplar resistance to Septoria canker in the United States

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Populus nigra (black poplar) is an important parent in hybrid crosses in the north central region of the United States. Sphaerulina musiva causes stem cankers and leaf spots on hybrid poplar impacting plantations across North America. In order to characterize the variability in resistance of *P. nigra* 47 genotypes from seven locations were inoculated with six different isolates of S. musiva. Host responses were compared using cankers cm⁻¹ (CPC) and disease severity score (DSS; 1 resistant - 5 susceptible). For CPC the majority of the variation (72%) in resistance was explained by location (P < 0.001) followed by genotype(location) (28%). In contrast, for DSS the majority of the variation (51%) was explained by genotype(location) (P < 0.001) followed by location (26%) with a significant isolate effect (P = 0.034) and a genotype (location) by isolate interaction (P= 0.004). The differences between CPC and DSS are likely due to how they measure resistance. CPC measures infection frequency, by counting lesion number, whereas DSS evaluates pathogen development following infection, based on lesion size. In a second experiment a subset of 12 genotypes were inoculated with a bulk spore suspension of all six isolates. Significant correlations between the two experiments for both disease severity parameters, CPC (r=0.89) and DSS (r=0.93), were found. The CPC and DSS values between a genotype inoculated with a single isolate and the same genotype inoculated with the bulk spore suspension were strongly correlated indicating that a single virulent isolate may be sufficient for preliminary disease resistance screening.

Using molecular tools to assess establishment and spread of the biological control agent of chestnut blight

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Cryphonectria parasitica, the causal agent of chestnut blight, is one of the best-known invasive fungal pathogens in forest ecosystems. Following its introduction, the pathogen caused a dramatic disease epidemic both in Europe and in North America. In many European regions, however, chestnut stands recovered thanks to the appearance and spread of natural hypovirulence. Hypovirulence is caused by the mycovirus Cryphonectria hypovirus 1 (CHV-1) which reduces virulence and sporulation of the infected fungal strain. This mycovirus has no extracellular form and can be spread either via asexual C. para*sitica* spores (vertical transmission) or through hyphal anastomosis between vegetatively compatible mycelia (horizontal transmission). The use of CHV-1 as biocontrol agent of chestnut blight has rapidly fascinated plant pathologists. Success and sustainability of the biological control of chestnut blight with artificially-introduced hypovirulence are, however, still controversial. On one hand, therapeutic treatment of individual cankers is mostly successful. On the other hand, establishment and spread of artificially-introduced viruses at the population level are more problematic and seem to depend on several factors. In my talk I will present how molecular markers (i.e. microsatellites for C. parasitica and SNPs for CHV-1) can be successfully used to follow the spread of the artificially released biological control agent and its fungal carrier in a chestnut stand.

Oral presentations

Tuesday 9 June

ASH DIEBACK

GREMMENIELLA

FOLIAR DISEASES ON CONIFER TREES

What makes the ash dieback fungus Hymenoscyphus fraxineus pathogenic?

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Recently, a new, invasive fungal pathogen (*Hymenoscyphus fraxineus*) affecting common ash (*Fraxinus excelsior*) has emerged as a serious forest health problem in Europe. The fungus is believed to be native in eastern Asia where it is acting as an endophyte in *F. mandschurica*. We have sequenced the genomes of *H. fraxineus* and its European congeneric *H. albidus* and also analysed gene expression following inoculation into ash trees. In our analysis we have identified gene families expanded in these species compared to other members of Helotiales. *H. fraxineus* has an active gene cluster likely to be involved in secondary metabolism that is non-functional in *H. albidus*. Differences in gene expression between the species when inoculated into ash will be discussed in light of the contrasting interaction outcomes with the host tree.

Evaluation of fungicides for control of ash dieback in forest nurseries

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Hymenoscyphus fraxineus (anamorph *Chalara fraxinea*) is the cause of ash dieback in many parts of Europe including the Czech Republic. This pathogen is responsible for extreme decline in ash production in nurseries. The use of effective fungicides could reduce losses and assure the production of healthy planting material.

In previous in vitro experiments, 26 fungicides at six concentrations were evaluated for their effect on mycelial growth. The most effective fungicides were Baycor (a.i. bitertanol), Discus (kresoxim-methyl), Falcon (spiroxamine, tebuconazole, triadimenol), Flint plus (captan, trifloxystrobin), Horizon (tebuconazole), Ortiva (azoxystrobin) and Zato (trifloxystrobin).

The in planta experiments were conducted in three forest nurseries in 2013/2014. In each nursery, at least 120 healthy saplings for each fungicide were used. Five fungicides which were evaluated as highly effective in *in vitro* experiments, were tested – Discus, Horizon, Ortiva, Zato and Score (a.i. difenoconazole). Two fungicide applications were carried out during the July 2013. In spring 2014, the percentage of symptomatic saplings was detected. The best results were obtained with Horizon 250 EW.

In 2014/2015 in planta tests were established in one forest nursery (42 experimental plots with dimensions of 150 x 120 cm). Six fungicides were tested – Discus, Horizon, Zato, Dithane (a.i. mancozeb), Mythos (pyrimethanil) and Talent (myclobutanil). The timing of applications was carried out according to the knowledge of the maturation period of the fruiting bodies. This test will be evaluate in spring 2015.

Spore dispersal of Hymenoscyphus fraxineus in the Czech Republic

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Our targed is description of *Hymenoscyphus fraxineus* spore dispersal in order to examine its biological relation to climatic conditions. For this experiment four infected localities in different parts of the Czech Republic (CR) were chosen. Two of the localities were situated in urban areas (around 400 and over 500 m a, s, l, in north-east and south-west of the CR), and the other two in forests (170 and 470 m a. s. l. in south-east of the CR). In every locality an automatic volumetric spore trap and climatic station were installed to continuously sample the aerospora. In few cases, a rotating arm spore trap was also used to obtain 48-hour air samples and to compare the efficiency of the two types of spore traps. To cover the known period of H. fraxineus spore release, the air sampling lasted from the beginning of June to the end of September 2014 in three of the localities. In the fourth one the sampling period was from the beginning of April to the beginning of November 2014 to reveal less probable spores' presence. The samples were divided into sections per days, from which the H. fraxineus DNA was extracted and quantified by specific real-time PCR with dual labeled probe. First results show a higher efficiency of the rotating arm type, which might be caused by the higher volume of air sampled. Another preliminary result is the unexpected occurrence of spores in October, more than one month after the last occurrence of *H. fraxineus* apothecia.

Local spatial population dynamics in the Ash Dieback pathogen *Hymenoscyphus* fraxineus

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The fungal pathogen *Hymenoscyphus fraxineus* has been causing widespread decline of European ash (*Fraxinus excelsior*) across Europe since the early 1990s. Recent genetic investigations of *H. fraxineus* across Europe found weak population genetic structure. While these studies focused on *H. fraxineus* isolates collected regionally and throughout Europe, finer scale resolution at the forest stand level has been lacking. At this local level, forest pathogen dynamics can be studied in the context of tree species composition. Diverse tree species mixtures can mitigate pathogen damage and limit spread compared with pure stands. In this study, the population structure of *H. fraxineus* was investigated using 14 microsatellite markers to genotype the fungus directly from petioles. Forest stands of varying tree species mixtures containing *F. excelsior* in Hainich National Park in central Germany were chosen to elucidate the local spatial population dynamics.

In vivo and in vitro characterization of the pathogenicity of Hymenoscyphus fraxineus: research in perspective of ongoing COST FP1103 FRAXBACK Action

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Currently, severe dieback of *Fraxinus* spp. is observed in most European countries. This is an emerging disease, which results in massive tree mortality, threatening the existence of Fraxinus over the continent. It is caused by Hymenoscyphus fraxineus, alien and invasive fungus, origin of which remains unknown. Many European countries have national research programs on *Fraxinus* dieback, focusing on numerous aspects of the biology and ecology of the disease, but the activities are scattered. Aim of the FRAXBACK is, through sharing and synthesis of available knowledge, generate comprehensive understanding of Fraxinus dieback phenomenon, and to elaborate state of the art practical guidelines for sustainable management of *Fraxinus* in Europe. Here, the pathogenicity of the invasive pathogen Hymenoscyphus fraxineus was evaluated in vivo and in vitro. As a significant correlation was found between the mycelial growth rate and colour in previous studies (darker mycelia appeared to grow slower), we wanted to confirm if this result might be a regular pattern in the pathogenicity of *H. fraxineus*. Therefore, five bright fast, and five dark slow isolates together with five dsRNA-hosting isolates were chosen from the pathogen collection, and cultivated on five different nutrient media (PDA, MOS, MEA, AMEA, and PIL) at 23 °C during 6 weeks. The growth rate and colour were then analysed in a total of 375 cultures. In addition, slow and fast growing cultures were inoculated on two-year-old seedlings of three different tree species, Fraxinus excelsior, F. *americana* and *F. pensylvanica*. The lesion lengths formed on the seedlings were weekly measured and compared with the growth rate of the same cultures in vitro. This study improves our understanding of the behaviour and growth of this invasive forest pathogen.

Hymenoscyphus fraxineus on Fraxinus mandshurica in Far East Russia: genetic diversity, associated fungal community and evidence of endophytic lifestage

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Hymenoscyphus fraxineus, the ascomycete fungus causing large-scale dieback of common ash in Europe, was investigated on its native indigenous host, Fraxinus mandshurica, in Far East Russia. Leaves were collected from asymptomatic F. mandshurica trees at three location in the Primorye forest region. The aims of the study were to i) determine the presence and behaviour of *H. fraxineus* on *F. mandshurica*, ii) describe its associated foliar fungal community, and iii) determine the specific structure of genetic diversity H. fraxineus between populations in East Russia and Europe. 454-sequencing of samples revealed 49 distinct fungal taxa, 12 of which were identified to species level. 96% of the sequence reads existed in 20 OTU (distinct taxa) belonging to six orders of Ascomycetes: Capinodiales, Pleosporales, Diaporthales, Helotiales, Hypocreales, Dothideales and an unknown Ascomycete, and to one order of Basidiomycetes: Tremellales. Members of *Capinodiales* and *Pleosporales* dominated the community in species richness. Many fungal organisms have similarity those inhabiting F. excelsior in Europe based on previous studies, although Mycosphaerella sp. which was clearly the most dominant species, and detected in nearly all samples, appears to be uniquely associated to F. mandshurica. H. fraxineus was detected from 33% of the total number of samples examined all three sites. The genetic diversity of the *H. fraxineus* population was significantly higher compared to European population. In contrast to Europe where H. fraxineus is an aggressive pathogen on F. excelsior, the fungus appears to be a benign associate of indigenous *Fraxinus* species within its native range, and displays endophytic behaviour. We propose that *H. fraxineus* has a biphasic lifestyle where in its endophytic stages establishes inconspicuous and at least transiently symptomless infections on F. mandshurica. Following leaf senescence, a lifestyle switch from endophyte to saprophyte occurs that facilitates decomposition of plant material.

Northward migration of Gremmeniella abietina from Italian peninsula

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Scleroderris canker caused by the ascomycetes fungus *Gremmeniella abietina* causes several damages on conifers species in the northern hemisphere. The disease has been recognized in Italy since the 1970s. The outbreaks were closely related to environmetal conditions. The fungus was found along the italian peninsula in two forms: Alpine and continental, on different pine hosts, growing under different environmental conditions. In the Alpine region this pathogen mainly affect *Picea abies, Pinus cembra, P. sylvestris* and *P.nigra*, while in the Mediterranean region it occurs on *P. pinea, P. pinaster, P. halepensis, P. nigra*. The damage typically involved the young plants in reforestation, but also on terminal shoots on adult pine trees. The climate changes of recent times have reduced the presence of the pathogen *G. abietina* in affected areas. The mild temperatures and less severe winters seasons, allowed the plants to recover. In many cases the pathogen was replaced by *Diplodia sapinea*, a more thermophilic fungal pathogen. These finding suggest that the effect of climate change play a relevant role on the incidence of Scleroderris canker, creating a new scenario to other pathogens mainly related to environmental stresses.

Two-year survival of *Gremmeniella abietina* conidia collected on branches left on the ground after pine harvesting

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In 1972, it was reported that viable ascospores and conidia of *Gremmeniella abietina*, North American race, were present on dead branches up to 10 months after they were killed. In Sweden, the survival period of conidia of *G. abietina*, European race, was reported to be over 18 months. In eastern Canada, we investigated the viability of conidia produced by *G. abietina*, European race, over a 2-year period. Infected branches with pycnidia were left on the ground in June 2006. Branches were sampled every month, except in winter, during this period. Conidia germination was monthly tested in four Petri dishes containing water-agar. Examination of 100 conidia was carried out at random for each plate after 60 hours. Conidia showed a very high rate of germination from July 2006 to August 2007. Very few pycnidia were detected in the fall of 2007. In May 2008, it was difficult to find any pycnidia and from the rare ones that were found, conidia germination was low (0 to 10%). In June and July of the same year, no pycnidia could be observed, the shoots being in an advanced stage of decomposition. In the light of these observations, it is recommended to delay pine plantation after two growing seasons following harvesting of diseased pine trees.

Antagonistic activity of fungal endophyte's filtrates against *gremmeniella abietina* infections on aleppo pine seedlings

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Biological control agents (BCAs) and among them some species of fungal endophytes, are potential substitutes for chemical pesticides in the control of plant diseases, because of their lower toxicity to human beings and environment. One mode of action of fungal BCAs is through their bioactive products, which can inhibit the growth of the pathogens by means of antibiosis. In this study, the effect of fungal filtrates from four isolates of endophytes was evaluated on the advance of the pathogen Gremmeniella abietina on two-year Pinus halepensis seedlings. Both the preventive and therapeutic treatments of the filtrates were studied by applying the filtrates before or after the pathogen inoculation, respectively. Six months after the inoculations, the symptoms of the disease started to appear (i.e. chlorosis, cankers and death of the plants) and the seedlings were processed. Since G. abietina is a necrotrophic fungus, the length of the necrosis produced by the pathogen was used as response variable in our experiment and was measured on every seedling. As a first step to explore the chemical composition of the fungal filtrates, a simple HPLC screening of UV-absorbing components was conducted. The results of the study showed that all fungal filtrates were able to reduce the advance of the pathogen as compared to the control seedlings. Low-molecular weight phenolic substances could be detected in some but not all filtrates, warranting further studies on the possible role of these compounds in fungal filtrates.

Interactions among temperature, a novel mycovirus and the conifer pathogen Gremmeniella abietina

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The occurrence of a novel RNA virus species was studied within the European race of Gremmeniella abietina. We examined 162 isolates originating from Canada, the Czech Republic, Finland, Italy, Montenegro, Serbia, Spain, Switzerland, Turkey and the United States. According to direct specific reverse transcription (RT) PCR screening based on the RNA-dependent RNA polymerase (RdRp) sequence, the virus was only present in Spain, but when quantitative RT-PCR (RT-qPCR) was applied, it was also found at a very low concentration in three isolates from Canada, Italy and Finland. To gain insight into the three-way interaction among temperature, mycovirus, and G. abietina, we performed an in vitro experiment with eight Spanish isolates (four infected and four uninfected) with four repetitions each. We assessed the virus expression based on the quantity of RdRp-encoding RNA by RT-qPCR and the G. abietina growth rate at 5, 15 and 20 °C. The presence of the virus had a significantly negative effect on the G. abietina growth rate at 5 and 15 °C. Moreover, the viral gene expression was demonstrated to be higher at higher temperatures in isolates in which there was a significant effect, but it did not have any influence on the growth rate of the infected isolates. Taken together, these results suggest that there could be a relationship between virus expression and temperature, although the role of this virus with respect to the more thermophilic nature of the Spanish population of G. abietina does not seem to be crucial. This is the first report in which qPCR is used in a population study of a mycovirus.

Notes on needle pathogens on larch in northern Finland

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Severe pathogens infecting larch (*Larix* spp.) needles are only a few, and all needle-inhabiting pathogens are less known and often occurring rather locally. One possible reason to the low incidence in the Nordic countries may be in the short co-evolution – larches recent history in northern Europe is only 300 years. Main emphasis of this presentation is to describe the recently found disease caused by *Hypodermella laricis*, which is new to Nordic countries, being able to cause severe outbreaks, defoliation and crown-thinning in the canopies of all ages of most planted larch species. Symptoms of *H. laricis* clearly differ from those of *Mycosphaerella laricina*, infecting *L. sibirica* at high latitudes for decades. The role of *Meria laricis*, *Lophodermium laricinum*, and various rust fungi as diseases are discussed.

In planta detection of needle pathogens in Pinus brutia needles

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Turkish Red Pine (*Pinus brutia* Ten.) is the most widely distributed and important forest tree species covering 24% of the total forested areas in Turkey. However, very little is known about its diseases. So far, Dothistroma needle blight (DNB) caused by *Dothistroma septosporum* and Diplodia shot blight has been of concern in some localities. On the other hand, information on the other important pathogens of pine species worldwide, such as *Lophodermium seditiosum* and *Lecanosticta acicola* is sparse for *P. brutia* in Turkey.

Species-specific primers were used to detect the presence of some important pathogens; *Dothistroma septosporum, Dothistroma pini, Lophodermium seditiosum* and *Lecanosticta acicola* directly in symptomatic and asymptomatic needles of *P. brutia*. Only *D. septosporum* and *L. seditiosum* were detected in the investigated needles. *L. seditiosum* occurred more frequently than *D. septosporum*. In symptomatic needles, detection frequency of *L. seditiosum* was higher compared with *D. septosporum*. However, detection frequencies of each pathogen did not significantly differ between the two categories of the needles (symptomatic vs. asymptomatic). Co-occurrence of the two pathogens in the same needle was detected in 27.8% of the investigated needles. Interaction between these two pathogenic fungi needs further investigations.

Fungal endophyte communities in needles differ between endemic and exotic pine species in nearby plantations in Turkey

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Conifer endophytes are presumed to be ubiquitous. More than a hundred different fungal species can be isolated from a single coniferous host. In conifer needles, fungal endophytes are restricted to discrete portions of tissues where they remain in a slow growing state, accumulating very little biomass. Restricted infections allow for many different species of fungal endophytes to occupy the same needle.

In this study, we investigated the endophytic fungal community in needles of *Pinus sylvestris*, *Pinus pinaster*, *Pinus nigra*, *Pinus taeda* and *Pinus radiata* in Kerpe Research Forest in İzmit in 2013. Ten trees of each pine species were sampled systematically. Healthy needles were sampled from the lower part of the canopy, from two equally spaced positions around the tree. Each needle was surface sterilized and cut into 0.5cm sections then individually placed onto 2% (w/v) Malt extract agar plates. From representative isolates DNA was extracted using the Qiagen DNeasy Plant Mini Kit. The nuclear 5.8 S rDNA gene and the two ITS regions flanked between the 18 S and 28 S rDNA genes were amplified, and the PCR products sequenced in both directions using the universal fungal primers ITS1 and ITS4. The obtained sequences were compared with known sequences in GenBank database using the BLAST algorithm.

The needle endophyte most frequently isolated was *Cenangium ferruginosum*, accounting for 30% of all fungal individuals, followed by *Cyclaneusma minus* (6% of all individuals). Eight main fungal taxa were identified: *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Diplodia*, *Pestalotiopsis*, *Rhizosphaera* and *Lophodermium*.

Invasive pathogens on pines (Pinus spp.) in Northern Baltics

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Two dangerous invasive pathogens of conifers, *Diplodia sapinea* and the causal agent of the quarantine Brown Spot Needle Blight *Lecanosticta acicola*, have lately started to spread and damage in Estonia.

Five years after the first finding of *D. sapinea* on exotic pines (2007 and 2012, respectively), it was registered on the native *Pinus sylvestris*. A year later, *D. sapinea* was registered in several *P. sylvestris* stands of southern Estonia. By the autumn 2014, *D. sapinea* was already found in few occasional points of northern Estonia whereas at the same time *P. sylvestris* stands in central and in most of the northern Estonia were uninfected. We tested by species-specific primers the presence or absence of *D. sapinea* (in latent phase) in several apparently healthy *P. sylvestris*. All the green, visibly healthy *P. sylvestris* shoots were uninfected.

First time *L. acicola* was documented on *Pinus ponderosa* in 2008 in Tallinn Botanic Garden. Up to 2012 it had repeatedly registered only in this restricted area. By today, it has been documented on exotic pine species already in 4 different localities around Estonia. Solely exotic and not our native Scots pine (*P. sylvestris*) were found damaged. Among exotics only *P. ponderosa*, *P. uncinata*, *P. mugo* and *P. mugo var. pumilio* were infected (the last apparently represents the worlds' first record). By 2014, only one mating type (MAT1-1) of *L. acicola* was repeatedly detected, recently also MAT1-2 was found for the first time.

Cedars under threat from Sirococcus spp.

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Atlantic cedar (*Cedrus atlantica*) is a species which could increase in importance to UK forestry with climate change, particularly on drier sites in southern and eastern Britain. In late autumn 2013 severe shoot blight and defoliation on Atlantic cedar was reported to the Tree Health Diagnostic and Advisory Service, England (UK). Affected branches displayed dead shoots, discrete sunken cankers and gum exudation. Isolations from the affected shoots and cankers were made onto streptomycin-amended malt extract agar (MAS) and a fungus was consistently isolated and identified as Sirococcus sp. based on conidium morphology. Amplification and sequencing of the ITS region, the actin gene and the translation elongation factor 1 alpha confirmed its identification as S. tsugae. From spring 2014 the number of symptomatic trees reported increased and all cases were confirmed using S. tsugae-specific primers and morphology. Pathogenicity tests were carried out on four year old cedar trees by spraying with a conidial suspension (5×10^6) conidia ml^{-1}). The symptoms were reproduced and S. tsugae was successfully re-isolated from inoculated plants confirming Koch's Postulates. The disease has been now detected in different locations in England, in Scotland and Wales and has also been confirmed on Tsuga mertensiana and on young T. heterophylla regeneration in the forest. This is the first report of S. tsugae in the UK and in Europe. Furthermore, another Sirococcus species, S. conigenus, is reported on cedar of Lebanon (Cedrus libani) causing severe shoot blight and shoot deformation. This is a new host-fungus association.

Damage by *Delphinella abietis* on subalpine fir in Norway may depend on the origin of the seed source

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Delphinella shoot blight caused by the fungal pathogen Delphinella abietis attacks several species of true fir (Abies spp.) in Europe and North America. Especially subalpine fir (A. lasiocarpa), one of the main Christmas tree species in Norway, is prone to the disease. The fungus kills current year needles, and in severe cases entire shoots. Dead needles become covered by numerous, black pseudothecia. Delphinella shoot blight has mainly been a problem in humid, coastal regions in the northwestern part of southern Norway, but probably due to higher precipitation in inland regions during recent years, heavy attacks were found in 2011 in a provenance trial of subalpine fir in Hedmark County in southeastern Norway. Clear differences in susceptibility between provenances were observed. Provenances with blue and greenish blue foliage were less attacked by D. abietis than green and blueish green provenances. In 2014, all 76 provenances in the trial were rated for symptoms of Delphinella shoot blight on a scale from 0 to 4 (0 = nodamage, 4 = severely damaged). The disease ratings were correlated with foliage colour recorded on a scale from 1 to 4 (1 =blue, 4 =green). The general outcome was that susceptibility to Delphinella shoot blight increased with increasing latitude of seed source in the Rocky Mountains, where the subalpine fir originates from, and it decreased with increasing altitude. This corresponds well with the observation that blue provenances, which are mainly found in higher altitudes in the south, are less attacked by this fungus.

Swiss needle cast foliage disease of Douglas-fir (*Pseudotsuga menziesii*) in the U.S. Pacific Northwest

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Swiss needle cast, an endemic foliar disease of Douglas-fir (*Pseudotsuga menziesii*), is caused by an ascomycete fungus *Phaeocryptopus gaeumannii*, and has reached epidemic proportions in the Northwest coast of US. Annual aerial surveys since 1996 have demonstrated that the number of hectares displaying SNC symptoms continues to increase with 2014 showing all time high of 237,000 ha. Because of sustained growth losses suffered by Douglas-fir, and the subsequent economic impact, a major research effort aimed at combating this disease has been initiated. Areas of research have included fungal biology and epidemiology, silvicultural treatments, genetic tolerance, predictive disease severity models. Numerous studies and predictive models have confirmed the association of spring leaf wetness and warm winter temperature with increased fungal germination and development. Although conventional silvicultural treatments have been applied within SNC-infected stands (thinning, fertilization, vegetation management, genetic tree improvement), disease symptoms have generally remained unaffected. Nevertheless, use of SNC-tolerant stock or thinning can improve the likelihood of infected stands reaching merchantable size in an acceptable amount of time. Foliage retention, the index of SNC infection intensity most highly correlated with growth loss. has been successfully predicted using seasonal climate variables. Application of future climate scenarios to this model provide an uncertain prognosis for the future. A new network of 100 plots is being installed throughout the Oregon and southwest Washington Coast Ranges as a means of monitoring future disease impact and providing a framework for additional studies aimed at understanding this most dastardly disease.

Reproductive isolation and population structure in the Douglas-fir Swiss Needle Cast (SNC) pathogen, *Phaeocryptopus gaeumannii*, in the U.S. Pacific Northwest

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The Douglas-fir foliage disease Swiss needle cast is caused by an ascomycete fungus, Phaeocryptopus gaeumannii. It has its greatest impacts in the U.S. Pacific Northwest forestry industry where it causes defoliation and growth reduction resulting in timber loss. It currently affects approximately 586,000 acres in Oregon. For this study, a total of 803 P. gaeumannii isolates were collected from foliage from 15 sites in the western Coast Range in Oregon and Washington. To date, 446 of these isolates have been genotyped at 9 nuclear microsatellite loci. Population genetic analyses suggest that the pathogen occurs in two reproductively isolated sympatric lineages with high genetic diversity at the level of the site. The distribution of the two lineages appears to be correlated with disease severity as assessed by aerial survey, and a strong east-west geographic trend in the proportions of each lineage suggests an influence of climate. This project aims to describe the influences of genetic diversity and gene flow on the population structure of this fungal pathogen, and determine the degree to which these factors influence the distribution of disease on the landscape scale. A thorough understanding of these factors is essential to the efficient mitigation of the disease impacts and their effects on forest communities and the Douglas-fir timber industry.

Oral presentations Wednesday 10 June Foliar diseases in broadleaved trees PINE PITCH CANKER DECLINE DISEASES MISCELANIOUS DISEASES

Impact of boxwood blight pathogens; Cylindrocladium buxicola and Pseudonectria buxi on Buxus balearica and Buxus sempervirens

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Impact of Cylindrocladium buxicola and Pseudonectria buxi (syn: Volutella buxi), and their co- infection on Buxus sempervirens and Buxus balearica were tested. Detached stems with leaves of the boxwood plants were inoculated with the conidia suspensions of the pathogens. Disease development was monitored 3, 7, 11, and 14 days post infection through visual assessment of infection levels using a 0-4 scale. Foliar symptoms were observed already 3 days after inoculation. Leaf symptom development was quickest on C. buxicola infections and slowest for P. buxi. Defoliation started on the C. buxicola inoculated plants 7 days after inoculations, and 11 days after on those inoculated with P. buxi. No defoliation observed on co-inoculated plants. Seven days post inoculations severe infection incidence accelerated on C. buxicola, and co-inoculated plants, yet remained stable on P. buxi inoculated plants afterwards. C. buxicola affected the plants more severely. However, its impact was not significantly different from the co-infection of C. buxicola and P. buxi. Portion of severely infected leaves was lowest on P. buxi inoculated plant material. Accordingly, co-infection of C. buxicola and P. buxi did not increase the disease severity of C. buxicola infection in our study. The susceptibility of B. balearica and B. sempervirens did not differ significantly to the blight pathogens and to their co-infection.

Dutch elm disease in Baltic sea island of Gotland

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The island of Gotland (Sweden) possesses the largest (more than one million trees) wild population of elms (mainly *Ulmus minor*) in northern Europe which until recently was not affected by Dutch elm disease (DED), caused by fungi from the genus *Ophiostoma*. In 2005, DED was for the first time observed in north-eastern part of Gotland and in the following years it rapidly spread in all directions, generally following major distribution of elms. Implementation of intensive control measures including ground surveys and geographic information system (GIS) mapping of DED-diseased trees, their harvesting and destruction, and stump treatment with the herbicide glyphosate allowed to reduce the source of infection and to hinder otherwise very rapid spread of the disease.

Scolytus multistriatus, the smaller European elm bark beetle, is a vector for DED in Gotland. Another aim of our work was to monitor flying intensity of *S. multistriatus*, collect beetles and, by using the next generation sequencing, to assess communities of vectored fungi, in particular focusing on *Ophiostoma* spp. Beetle sampling was carried out at two different sites of the island. In total, 50 pheromone traps were placed at each site and checked weekly during June-August. "Sticky" traps were used allowing to collect each beetle individually. During three last years, a total of 177 beetles were trapped: 6.2% of them in June, 76.8% in July and 16.9% in August (difference significant at p<0.007).

Direct sequencing of ITS rDNA from the beetles revealed the presence of 1589 fungal taxa, among which virulent DED pathogen *Ophiostoma novo-ulmi* was the second most common species, comprising 9.0% of all fungal sequences. *O. ulmi*, less virulent DED pathogen, was also detected but only in one beetle collected in first sampling year 2012. There were 13.0% of the beetles infested with *O. novo-ulmi* in 2012, 4.0% in 2013 and 27.7% in 2014. In conclusion, the study showed that the proportion of *S. multistriatus* vectoring *O. novo-ulmi* has increased in the recent season, and that population of *O. ulmi* in Gotland has seemingly ceased to exist.

Preliminary analyses of ITS sequence variation among Marssonina leaf spot and shoot disease pathogens of poplars

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Fungi in the anamorphic genus Marssonina Magnus (ascribed to the teleomorphic genus Drepanopeziza (Kleb.) Höhn, family Dermateaceae) cause leaf spot and shoot diseases of naturally occurring and cultivated *Populus* species and hybrids. Defoliation, dieback, and growth loss can limit utilization of susceptible, but otherwise productive clones. Recognition of species is based largely on morphology of conidia, which overlap in size and vary in shape; these characters can be influenced by factors including substrate. Pathogen identification, clarification of geographic distribution, improved understanding of disease biology, and knowledge of relative host species and clone resistance or susceptibility would be facilitated by molecular-based methods. ITS sequence data was obtained for a preliminary collection of isolates from symptomatic leaves from North America and isolates from the *Ribes* leaf pathogen *D. ribis* (Kleb.) Höhn. Phylogenetic analyses support placement of the majority of isolates into a single well-supported clade, likely M. brunnea (Ellis & Everh.) Magnus (Drepanopeziza tremulae Rimpau). Isolates in this clade originated from locations in the western Canadian province of British Columbia, the northcentral USA states of Michigan, Minnesota, Wisconsin, and southern states of Arkansas and Mississippi and from diverse hybrids including parents in P. deltoides, P. maximowiczii, P. nigra, P. trichocarpa, and the species P. alba. A small number of isolates from species P. balsamifera and P. alba in Minnesota and Wisconsin, respectively, were placed in two distinct clades. Additional collections and phylogenetic analyses, including relationships of the Marssonina leaf spot and shoot disease pathogens of poplars within the Dermateaceae, are underway.

Spatial distribution of sibling species causing European oak powdery mildew

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The availability of molecular markers has led to the recognition that many fungal plant pathogens are in fact complexes of sibling (cryptic) species. This raises questions about the comparative epidemiology and ecology of these closely related species : do they have the same epidemiological significance? how can the coexistence of species sharing the same resource be explained? We formerly showed that several closely related lineages of *Erysiphe* can cause symptoms of powdery mildew on oaks in Europe and that the two species most commonly found in France (E. alphitoides and E. quercicola) differ in their seasonal dynamics. Here we studied their spatial distribution, from a large regional scale to the leaf scale. We tried to identify the factors explaining these distributions (climate, source of primary inoculum, host species) and to relate the presence of the different species to disease severity. We found that E. quercicola is limited by very cold winters, is more frequent on seedlings than on trees and on *Q. petraea* than *Q. robur*. No link could be established between the relative frequency of E. quercicola and E. alphitoides in early spring and the disease severity at the end of the season. Whereas E. alphitoides and E. quercicola often coexist at a very fine scale, spatial differentiation is suggested to be more important between E. alphitoides and E. hypophylla. The preferential localisation of *E. hypophylla* on the abaxial (lower) leaf surface as effuse colonies was confirmed.

Evaluation of the antagonism produced by fungal endophytes against *Fusarium* circinatum in the field

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Pitch canker is a virulent disease of pines caused by the fungus Fusarium circinatum Nirenberg & O'Donnell. It produces damages in nurseries as well as as in adult trees in the forest. Symptoms in seedlings include damping-off and wilting of seedlings. On mature trees, the main symptom of pitch canker is a bleeding resinous canker in the stem or thick branches, although branch die-back also occur. The disease has no means of control at the present. The current restrictions on the use of chemicals and fungicides in the forest forces the research on alternative methods to reduce the damages caused by the pathogen. In this line, biological control using fungal endophytes is a promising and environmentally friendly strategy. Six isolates of fungal endophytes with good results in a previous in vitro antagonism experiment were inoculated in the field together with the pathogen on seedlings of the pine species Pinus radiata, P. sylvestris, P. pinaster, P. nigra and P. pinea, to check if they were able to reduce the damages caused by the *F. circinatum*. Two of the endophytes, *Chaetomium aureum* and *Alternaria sp.*, reduced the area under disease progress curve (AUDPC) of the *P. radiata* seedlings, and therefore they must be suitable to be used as biological control agents (BCAs).

Pityophthorus pubescens associated with Fusarium circinatum in northern Spain

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Fusarium circinatum, the causal agent of pine pitch canker (PPC), is a threat to Pinus ra*diata* plantations in Cantabria (northern Spain) due to the high susceptibility of this pine species. This pathogen has been reported to be phoretically associated with several bark beetle species worldwide. *Pityophthorus pubescens* (Coleoptera: Scolytinae) is a secondary pest in our study area attacking weakly trees. The aim of this study was to know the association between *P. pubescens* and *F. circinatum* in PPC affected plantations. For this purpose, funnel traps baited with (E)-pityol were established and twigs from infested trees were sampled to collect both, insects and plant material in pitch canker affected plots. A total of 263 beetles were collected from funnel traps from June to September 2010, 2011, 2012 and 2013. Moreover, 215 specimens were collected from 424 galleries within the twigs in 2012 and 2013. Insects and plant material were plated on culture media with the aim of isolating F. circinatum. The pathogen appeared on 1.05 % and 2 %of the collected insects in the funnel traps during 2010 and 2012 respectively. Regarding the collected twigs, F. circinatum was found in 3 galleries, although no isolates were obtained from the insects. This work shows a weak association of P. pubescens with F. circinatum in this study area.

Keywords: Pinus radiata, pitch canker disease, twig beetle.

Impact of seed transmission on the infection and development of pitch canker disease

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Pitch canker disease, caused by the fungus Fusarium circinatum, is restricted in Spain to the Atlantic forests, and its occurrence is a serious threat to Pinus pinaster and P. radiata stands. In nurseries, this disease causes pre- and postemergence damping-off as well as mortality of established seedlings. Disease outbreaks in nurseries are related with seed transmission of F. circinatum. The objectives of the research were to: (1) study transmission from adult trees of *P. radiata* to seeds; (2) determine effect of *F. circinatum* presence on seed germination and germling growth; (3) study fungus effect on pre- and postemergence damages; and (4) investigate fungus persistence in plants. Seed transmission was estimated on 8 plantations of *P. radiata* over 100 seeds sampled from each of 10 trees per plantation. F. circinatum was detected within or on seeds in percentages from 0 to 12%. The results of seed inoculation assays showed that internal or external fungal presence did not alter the germination percentage, affected only by the inoculation dose. Seedlings grown from inoculated seeds showed symptoms of wilting and dieback, and F. circinatum was recovered from all of them. More importantly, F. circinatum was also recovered from two symptomless seedlings at 475 days post inoculation. Postemergence damages were 70 and 20 % for high and low inoculum doses (106 and 104 spores/mL), while proportion of premergence damping off was only significant for high inoculum doses.

COST Action FP1406: Pine pitch canker - strategies for management of *Gibberella circinata* in greenhouses and forests (PINESTRENGTH)

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Gibberella circinata is a highly virulent pathogen damaging pines, causing damping-off in nurseries and pitch canker in forests. It was first detected in North America, since when the pathogen has spread into Central and South America, South Africa, Asia and, more recently, Europe. Gibberella circinata is now considered the most important pathogen affecting *Pinus* seedlings and mature trees in many countries globally; asymptomatic seedlings may be planted out, resulting in very serious losses in forests. Nevertheless, there has been little research on G. circinata in Europe to date and little information is available overall on host range in Europe, pathogen spread and disease control. The main aim of this Action is to establish a European-focused network to increase knowledge of the biology, ecology and pathways of spread of G. circinata, to evaluate the actual risk for European pine forests and plantations and examine the potential for the development of effective and environmentally-friendly prevention and, where necessary, mitigation strategies and to deliver these outcomes to stakeholders and policy makers. To that end, a multidisciplinary approach will be taken, including researchers, forest managers and policy makers from (initially) 27 countries focused on the common problem of pitch canker, making PINESTRENGTH highly innovative.

Abiotic factors involved in Pinus pinaster decline in the Iberian Peninsula

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Pinus pinaster Ait., is one of the most important Spanish forest species, for its protective value and its timber production. Maritime pine decline was observed in several forests in the centre of the Iberian Peninsula. There is an unusual transparency at the crown, small needles, foliage discoloration and early tree death which are characteristic decline symptoms associated with the high mortality rate with this species. At present there are no studies that relate this tree decline in the centre of the Iberian Peninsula with forest management, soil properties and climatic factors. The specific goals of this study are: (1) to obtain an order in the distribution of P. pinaster decline in central Spain in relation to groups of variables that defines the decline. (2) to understand the abiotic factors associated with the decline of this pine species. (3) to build a model that allows prediction of the state of tree health based on the influencing abiotic factors. In all, 27 circular plots of 15m radius which were located in the main areas of distribution of the species in the region have been sampled. It has obtained that the distribution of P. pinaster decline in the central-north of Spain is not widespread, but instead is localized in specific areas. The results show that the sampled plots are influencing by the forest structure and climatic factors.

Genomic analysis of bacteria associated with Acute Oak Decline

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Both species of native oak trees in the UK (Quercus robur and Quercus petraea) are affected by a Decline syndrome termed Acute Oak Decline (AOD). Black weeping patches on the stems of mature trees are the primary symptom of AOD and indicate the presence of necrotic lesions in the underlying tissue. Two newly described bacterial species, Gibbsiella quercinecans and Brenneria goodwinii are consistently isolated from necrotic lesions. It has been suggested that these bacteria have a central role in tissue necrosis, possibly via genome encoded pathogenicity islands, plasmids or phage encoded enzymes. The aim of this investigation is to identify the presence of genes associated with bacterial pathogenicity. This study used the Illumina MiSeq second generation and Pacific Bioscience third generation sequencing platforms, for the *de novo* assembly of Gibbsiella quercinecans and Brenneria goodwinii. Resultant assemblies were annotated using the RAST web server, PROKKA, a prokaryote specific annotation tool and the CAZy database. This genomic analysis has revealed a number of potential virulence factors. These include Plant Cell Wall Degrading Enzymes (PCWDE), numerous ironacquisition siderophores such as enterobactin, virulence mediating quorum sensing systems and assorted secretion systems. Similar studies of bacterial associated plant pathogens have indicated that the synchronous relationship of these genes in for example Pectobacterium carotovorum leads to maceration of host cell tissue. The annotated genes encompass a suite of virulence factors providing G. quercinecans and B. goodwinii with the tools to compromise oak tree health. A combination of second and third generation sequencing analysis has provided substantial evidence implicating Gibbsiella quercinecans and Brenneria goodwinii as potential pathogenic contributors to the Acute Oak Decline syndrome.

Monitoring forest diseases and syndromes: the case of Acute Oak Decline

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In recent years, in the UK, an atypical form of oak decline with distinctive stem symptoms has become apparent. Acute Oak Decline (AOD) develops faster than the longknown chronic form of decline (Denman et al., 2014). Evidence suggests that AOD is a consequence of the interaction between Agrilus biguttatus (Brown et al., 2015) and several species of bacteria (Brady et al., 2010; Denman et al., 2012). Study of this newly defined syndrome has involved a multidisciplinary approach to answer some of the many unknowns surrounding its epidemiology. Here we present a few of the key findings from two mapping and monitoring exercises. 1) At the stand level long term monitoring plots were established to investigate disease dynamics within the system. In addition to rates of transmission these data revealed a further process that regulates the rate of disease development. This process involves the growth of callus tissue over of lesions and larval galleries, resulting in the remission of lightly infected oak and offers hope that management and selection within infected stands can mitigate the impact of AOD. 2) At the landscape scale information initially collected through citizen science has been enhanced and verified through a national woodland survey. From these data it has been possible to estimate the risk of AOD occurrence across Great Britain using a method developed by Parnell et al., (2011). The survey data will now be used to inform future work relating the distribution to climate and biogeographical variables, especially those that influence water availability.

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Identification of bacteria associated with the acute oak decline syndrome in England using Next Generation Sequencing

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Outbreaks of acute oak decline (AOD) have been documented in England from 2006 and this condition is spreading through the Midlands and South East England. Both species of native oaks (Ouercus robur and Ouercus petraea) are affected. At Forest Research, certain bacteria were consistently isolated from symptomatic trees which are thought to cause necrotic patches in the bark and sapwood. To complement isolation efforts for identification of putative causative biotic agents and increase our understanding of bacterial communities associated with oak tissue in general, 5 different sites in England namely Attingham Wood, Runs Wood, Great Monks Wood, Stratfield Brake and Bisham Wood were chosen for this study. Samples of outer bark, inner bark, sapwood and heartwood were taken from healthy oak and trees with symptoms at varying stages of the syndrome. Furthermore, larval galleries were included in the study. All samples were subjected to molecular analysis using Next Generation Sequencing (NGS). After DNA extraction and purification the V3-V5 fragment of the 16S rRNA gene was amplified followed by pyrosequencing. The dataset was analysed by QIIME followed by multivariate statistical methods to identify patterns in bacterial communities between the different sites and syndrome stages. Overall, the composition of bacterial communities differed greatly according to the geographical location of their hosts. Within each site the composition of the bacteria associated with symptomatic tissue varied between advanced stages of the syndrome and healthy tissue. Key players in healthy and symptomatic tissue were identified and included members of the Gammaproteobacteria and Firmicutes.

Simulating key elements of Acute Oak Decline to produce signs and symptoms in log tests

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Acute Oak Decline (AOD) is a newly defined distinctive condition in Britain, identified by weeping patches and D-shaped exit holes of the native buprestid Agrilus biguttatus on stems and larval galleries in the phloem and sapwood. Novel bacterial species are associated with the bark and sapwood necroses which led to the hypotheses that (i) the bacteria spp. Brenneria goodwinii (Bg) and Gibbsiella quercinecans (Gq) have a role in causing necrosis and degradation of tissue beneath the stem bleeds, and (ii) interaction between A. biguttatus and these bacteria will lead to typical AOD symptoms. Experiments to test the hypotheses are being pioneered and preliminary findings will be presented. An inoculation trial was carried out on 20 cm diameter logs of O. robur set out in a randomised split-plot design (6 blocks). Twelve treatment combinations using Bg, Ga, A. biguttatus eggs and A. biguttatus larvae were applied to the logs singly or in combination through wounds made with a 5 mm cork borer. Controls used water only. At the end of the experiment lesions were exposed and lesion areas calculated using APS Assess (V1) image analysis. Tissue from the dead-live junctions of each lesion was plated onto PYGA culture media and Bg and Gq were re-isolated from bacterial inoculations. Some parts of the experiment suffered contamination but the experimental design was robust enough to allow hypothesis testing using residual maximum likelihood (REML). Significant differences between the treatments (p<0.001) occurred, with 3 significant effects namely, presence / absence of Bg; presence / absence of A. biguttatus egg or larva; and their interaction (all p<0.001) identified. Combined treatments gave the largest lesion areas i.e. (Bg+Gq; Bg+Gq + A. biguttatus | arva, Bg+Gq + A. biguttatus egg and Bg + A. bigut*tatus* egg) indicating the cumulative effect of the different agents on lesion area. Bg was common to these treatments and it appears that it may be the most important necrogen in AOD. Application of either Bg or Gq caused similar sized lesions that were not significantly different to controls. Larvae on their own gave the smallest lesions. Gibbsiella only had a significant effect on lesion size if it was combined with B. goodwinii. Thus preliminary results suggest that Bg is essential to the development of AOD; A. biguttatus (eggs or larvae) are essential for producing the galleries that are a diagnostic sign of AOD; and that Bg in combination with A. biguttatus eggs or larvae causes the symptoms of AOD, whereas Gq appears to enlarge lesion areas when combined with Bg but cannot cause AOD symptoms in combination with A. biguttatus eggs or larvae.

Evolutionary adaptations of *Pseudomonas syringae* pv. *aesculi* to the European horse chestnut

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Pseudomonas syringae pv. aesculi (Pae) is the causal agent of the recent epidemic of bleeding canker disease of European horse chestnut (Aesculus hippocastanum) in northwest Europe. The main symptoms are bleeding bark cankers on the stem and branches which can lead to tree mortality. A comparative genomics study of 64 isolates of 33 pathovars of *P. syringae*, including 20 pathovars from woody hosts, was conducted within a phylogenetic framework to i) elucidate the history of gene acquisition along the lineage leading to the Pae pathovar, ii) identify genes with a potential role in the adaptation of P. syringae on to woody hosts and iii) identify genomic differences among six Pae isolates that may indicate potential routes of transmission in Europe. Adaptive shifts from herbaceous to woody hosts in the *P. syringae* complex were strongly correlated with uptake of specific sets of genes as a result of horizontal gene transfer. Genes of potential interest in the Pae lineage include a D-xylose operon that may confer an ability to utilise a hemicellulose carbon source present in woody species, the acquisition of two pathways involved in the degradation of phenolic compounds and several type III effectors. Very little nucleotide diversity was found among European isolates of Pae, suggesting a single and recent introduction of the bacterium into the continent. This approach provides novel insights into the processes of adaptation of Pae to a woody host. Functional analyses are now required to elucidate the role of these genetic pathways in the infection process.

Inoculation tests of Phytophthora alni and P. plurivora on alder seedlings

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The ecological importance of riparian forests because of its conservation role, maintaining biodiversity and stabilizing riverbanks is well known. However, a severe decline of the riparian alder population associated with *Phytophthora* species has become an important issue in Europe, and particularly in Spain. The aim of the work reported here was to compare the pathogenicity of P. alni and P. plurivora on A. glutinosa seedlings. This study demonstrated that P. plurivora caused as much damage as P. alni. In fact, P. alni caused mortalities ca. 30 %, whereas P. plurivora killed ca. 50 % of seedlings. Likewise, no differences in lesion lengths and biomass allocation were found between P. alni and P. plurivora. This study confirms P. plurivora, one of the most common Phytophthora species in European nurseries, is a major threat to alders and riparian forests sustainability and, therefore, special attention should be paid to the risk that this pathogen escapes from nurseries to the field. Nowadays, alders and many other trees and shrubs species are used for hydrological restoration and recreation areas in the vicinity of rivers, which increases the risk of *P. plurivora* spread from nurseries to the field via latent, asymptomatic infections. Further studies and surveys are also essential to unravel the role that *P. plurivora* is playing in alder decline individually as well interacting with P. alni.

The use of mycoviruses in forest disease management

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About thirteen families of mycoviruses have been described with 80% of mycoviruses catalogued. Additionally, their evolutive relationship with fungi is still unclear. Mycoviruses genome can be formed by single or double strains of RNA (ssRNA/dsRNA) or double strain of DNA (dsDNA). The effect of mycoviruses in fungi can range from cryptic state (asymptomatic), hypervirulence promotion or hypovirulence effect in their host. Horizontal transmission of mycoviruses can be made difficult by the presence of different vegetative compatibility types (vc) and mating types (MAT's). Mycoviruses-mediated hypovirulence has proved useful in biocontrol of many plant diseases like Chestnut blight (causal agent *Cryphonectria parasitica*). Development of this biological control tool in other forest pathologies requires a preliminary deep knowledge in viral symptomatology and transmission limitations. We perform a review about mycoviruses application and future use perspectives on forest diseases biological control as well the advances in mycoviruses applied research for forestry, landscape and woody culture diseases.

Decay of living western redcedar: overview of an information synthesis and new research in progress

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Despite western redcedar (WRC; *Thuja plicata*) wood having a reputation for decay-resistance, live WRC is well-known for its extensive heartwood decay, which negatively impacts the yield of harvested trees. To reduce WRC volume losses to decay organisms we need to better understand infection dynamics, i.e., 'who' the key decay organisms are, how and when they get started in living WRC, what their incidence is, how they interact with forest management activities and what their actual impact is on volume.

A literature review we conducted in 2013/14 indicates that more than two dozen different species of decay fungi occur on living WRC trees, with the following five proposed to be the most common and/or causing the most significant damage to coastal and interior WRC: *Phellinus weirii*, *Physisporinus rivulosus*, *Perenniporia subacida*, *Porodaedalea pini*, and *Postia sericeomollis*. Research we have initiated and propose to conduct in collaboration with Academic, Government and Industry colleagues to better understand decay dynamics in WRC include inoculation and screening trials, identification of decay-caused volume losses, and development of best practices to manage this valuable species.

Inter- and intra-specific variation in susceptibility to dothistroma needle blight in Britain. How susceptible are *Pinus sylvestris* and *Pinus contorta*?

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Dothistroma needle blight (DNB), caused by Dothistroma septosporum, is a serious disease in British pine plantations. Natural infection (NI) and artificial inoculation (AI) experiments were conducted to evaluate the susceptibility of Pinus sylvestris and Pinus contorta to DNB, relative to Pinus mugo, Pinus muricata, Pinus nigra ssp. laricio, Pinus nigra ssp. nigra and Pinus ponderosa. A range of P. sylvestris and P. contorta provenances were included in experiments to simultaneously investigate intra-specific variation within these host species. In NI experiments, 20-30 12-month-old seedlings of each species/provenance were planted adjacent to symptomatic mature trees at two Scottish sites. In the AI experiment, eighteen 16-month-old seedlings of each species/provenance were inoculated with a D. septosporum conidial suspension and maintained under optimum conditions for *D. septosporum* infection and development. In the NI experiments, the most DNB-susceptible species were P. muricata and P. ponderosa, while the least susceptible species were P. contorta and P. sylvestris. Pinus nigra spp. nigra and P. nigra spp. laricio had intermediate DNB-susceptibility. In the AI experiment, there was no evidence for inter-specific variation in DNB-susceptibility. Intra-specific variation in DNB susceptibility within either P. contorta or P. sylvestris was not observed in either experiment. In a further AI experiment with 24-month-old P. sylvestris and P. contorta seedlings, the virulence of three Scottish D. septosporum isolates (haplotypes) was examined. There was no significant interaction between isolate and relative susceptibility of pine species/provenances. Furthermore, no between-isolate variation in virulence was observed.

New fungal associates of the wood boring beetle *Platypus cylindrus* F. (Coleoptera: *Curculionidae*) with special focus on *Raffaelea montetyi* fungi in Tunisian cork oak stands

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Among the ambrosia beetles (Coleoptera: Curculionidae) infesting cork oak stands in Tunisia the most important is *Platypus cylindrus*, the cork oak pinhole borer. As all the xylomycetophagous insects, *P. cylindrus* ensures the transportation and storage of fungal propagules in their mycangia, especially species of Ophiostomatales. Only the genera Ophiostoma (s.s) and Raffaelea were reported to be associated with P. cylindrus namely in Portugal, Algeria and France. In Tunisia, the only research dealing with fungi associated with insects was led by Ben Jamâa in 2007 which identified Leptographium wingfieldii. Ophiostoma minus and O. ips isolated from Tomicus piniperda and Orthotomicus erosus in Tunisian pine forests, however, in cork oak stands little is known of these fungi. The present study was conducted in 2011 and focused on ophiostomatoid fungi associated with the cork oak pinhole borer in Tunisia. Fungi were isolated from insects and their galleries belonging to five different cork oak forests in the western-north of Tunisia. We identified 50 isolates of ophiostomatales belonging to Raffaelea genera. The specific identification was based on morphological approaches and on sequence data obtained from the ITS and LSU rDNA regions of the fungal species collected. Raffaelea montetyi is now reported for the first time in Tunisia as a representative of ophiostomatoid fungi.

Oral presentations

Friday 12 June

NURSERY PEST AND DISEASES

Soil fumigation techniques used in the production of forest-tree seedlings in the southern United States

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In the southeastern United States, fumigation with methyl bromide (MBr) has been the most commonly used method for producing high quality, pest-free forest-tree seedlings in an environment that is conducive for soil-borne pathogens, nematodes, and weeds. As a result of the Montreal Protocol, the production and use of MBr was to be incrementally phased out beginning in 2005. Included in this process are exemptions allowing for continued use and testing of fumigants with the goal of finding an alternative that is economically feasible and efficacious. Testing by the Southern Forest Nursery Management Cooperative (SFNMC) has shown that, although there are alternatives to MBr, they are not as efficacious. Any choice of currently available alternatives will most likely require an increase in pesticide use to compensate for alternative short-falls. The effects of all alternatives following 4-5 crop rotations without methyl bromide are unknown. Currently, recommended alternatives vary in their effectiveness from one nursery to another due to soil type and climate. The most significant development in soil fumigant research in the last 5 years has been the availability of high barrier plastics that will allow lower fumigant rates to be used. The most efficacious alternative for forest seedling nurseries in the southern United States is one that contains a significant percentage of chloropicrin as its active ingredient.

First report of *Rosellinia* spp. on seedling of *Pinus sylvestris* and *Picea abies* during cold storage in a forest nursery in Sweden

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Several Swedish forest nurseries use cold-storage (0° and 100% humidity) of conifer seedlings during winter. In 2012 and 2013 many seedlings as well as surrounding frames became covered with a white-brownish mycelium in one nursery. At the same time seedlings were also infected by *Botrytis cinerea*. Sequencing of the ribosomal DNA region from one pure isolate showed that the fungus belonged to *Rosellinia* spp., while sequencing directly from the mycelium on the seedlings revealed *Rosellinia nectroides* (or *Rosellinia ascondita*). It was possible to re-infect small conifer seedlings during laboratory conditions with mycelia isolates. We also tested the effect of some fungicides in an *in vitro* experiment on agar plates and the mycelium was sensitive to Azoxystrobin in concentrations from 10 ppm. The results will be discussed.

Rhizosphere fungi of nursery grown conifer seedlings

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Forest nurseries in Sweden produce ca. 358 million seedlings of Pinus sylvestris and Picea abies annually. Fungi represent the largest microbial component in the rhizosphere and may significantly affect health and consequently quality of the seedlings. The aim of this study was to assess fungal communities colonising roots and growth substrate of healthy-looking conifer seedlings from nine forest nurseries situated in northern, central and southern regions of Sweden. In each nursery, 100 seedlings of each tree species were collected. DNA was separately isolated from part of the root system of each seedling and from the growth substrate, amplified using ITS rDNA as a marker and 454-sequenced. Clustering of 169844 high-quality sequences resulted in 839 non-singleton contigs, at 98.5% similarity level representing different taxa. The most common taxa were Hyphodiscus sp. (19.9% of all sequences), Thelephora terrestris (16.2%), Cryptococcus terricola (12.3%), Leucosporidium drummii (10.0%) and Atradidymella muscivora (7.7%). Results demonstrated that rhizosphere environment in forest nurseries is inhabited by species-rich fungal community which is composed of different functional groups of fungi. Patterns of fungal communities observed in different tree species, forest nurseries and geographical regions will be discussed.

The strategic occupation of processionary moth *Thaumetopoea pityocampa*in the semi-arid Aleppo pine forests (Algeria)

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Aleppo pine forests in semi-arid are sensible to the processionary moth. The examination of 400 trees in natural and plantation forests infer that the rate of defoliation showed a significance variable in the plantations prospected, 25 to 50% for Moudjebara and from 10 to 25% for Tokerssan. In natural forests, it not exceeding 10%. The defoliations by processionary are variable in aegis of environmental conditions and in the dynamics of these populations. Frequency analysis of nests number counted per tree shows a very highly significant (p=0.0001). The concentration of nests is noted in trees that their length 4 to 6 meters with 3 to 5 nests per tree in Tokerssan situated in the north position. In the south, plantation of Moudjebara, the trees between 3 to 4 meters have significantly nests with 2 to 5 nests per tree. The distribution of nests in natural forests showed importance in the trees ranging from 3 to 5 meters, about 1 to 3nests per tree. This study highlighted the presence of plantation near natural forests facilitated the migration of adults from their origin sites where there are repeated treatments periodically in to the monoculture plantations which offered favorable conditions. The directions change of the insect indicate that the age, the exposure and the situation of forest enter in the choice of nests construction and in their strategy of occupation. Diameter measurement of nests taken at the altitude of 1300m showed that nests are longer and larger than those at 1200m where larvae number are less.

Poster presentations

Aneurinibacillus migulanus Nagano controls Dothistroma Needle Blight of Pine

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Dothistroma needle blight (DNB), caused by *Dothistroma septosporum* and *D. pini*, causes extensive damage to pine globally, wherever these species grow, with severe impacts on forest ecosystem productivity. There is an urgent need to find effective methods for disease control and management. Current methods include pruning and thinning of stands to reduce humidity, planting of less susceptible genotypes of host species and application of copper-based fungicides to forest stands.

Phytosanitary regulations demand the production of clean planting stock by forest nurseries. The possibility of using biological control to reduce the impact of DNB in nurseries, however, has received little attention.

Bacteria in the genus *Aneurinibacillus* have potential in biological control due to production of antimicrobial metabolites (e.g. gramicidin S; GS). The aim of the work reported here was to compare potential of two strains of *Aneurinibacillus migulanus*, strains Nagano and NCTC 7096, to provide control of DNB on *Pinus contorta*.

Two-year old *P. contorta* seedlings were treated with either *A. migulanus* Nagano or NCTC 7096 suspension. Plants were inoculated with *D. septosporum* condial suspension 24 hours later and incubated in an incubation chamber for seven weeks.

Treatment with *A. migulanus* Nagano significantly reduced the attachment and germination of *D. septosporum* conidia on needles after 7 and 14 days, and DNB severity after 7 weeks, compared to treatments with NCTC 7096 or controls.

The results are discussed in relation to the importance of producing clean nursery stock for outplanting in the forest.

Outbreak of needle blight diseases in *Pinus radiata*: emerging forest diseases in the North of Spain

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Tree damage caused by forest pathogens is influenced by the fungal strain nature, host resistance, and environment. Changes to host or environmental conditions can increase the incidence and severity of emerging infectious diseases. Until recently, pine needle blight diseases have had only minor impacts on native and exotic forest trees in the North of Spain. Over the past five years however these pathogen species have spread widely and caused severe defoliation and mortality in exotic plantations of *Pinus radiata*. We initiated an evaluation of specific symptoms and damage associated with these diseases, identified the main causal agents and to define the disease impact and establish a basis for monitoring and controlling their spread. Surveys in 202 locations showed that these symptoms are rather common. Infections result in premature defoliation and hence reduction of photosynthetic surface and tree vigor in young and adult trees and consequently wood volume losses. Over several years, these recurrent damages result in branch and tree death. The estimated average of disease incidence in plantations was $70.82 \pm$ 26.51%, values of severity ranged from 5% to 95%. Morphological characteristics of the fungus and molecular identification results were consistent with those of Lecanosticta acicola (Thün) Syd. and Dothistroma spp. Sustainable strategies of prevention and integrated control are suggested to reduce the increasing disease impact.

Selection and testing *Fraxinus excelsior* genotypes for resistance against *Hymenoscyphus fraxineus* in Sweden

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Since 2001, the invasive ascomycete fungus Hymenoscyphus fraxineus has resulted in a serious population decline of common ash (Fraxinus excelsior) in Sweden. Ash is an important species among the noble broadleaved trees available to forest management in Sweden. Thus, the newly observed ash dieback is a severe setback for noble tree forestry. In 2010, F. excelsior was among the first trees species added to the Swedish Red-List with vulnerable status considered at high risk of extinction in the wild. A significantly large number (254) of other fungal, insect and lichen Red-Listed species also depend on ash which suggests that this disease can have wider community impacts causing a cascade of species declines or extinctions. Recent studies have shown that a small proportion of the natural population of *F. excelsior* in Europe has better disease tolerance. Here we report on several activities aimed at selecting and testing F. excelsior genotypes including: 1) mapping, identifying and selecting hundreds of disease-tolerant F. excelsior from wild populations, 2) testing symptom development and screening resistance in a select number of F. excelsior families using an effective inoculation technique for ascospore infections in a controlled climate chamber, also for identifying critical defensive traits associated with resistance, and 3) to estimate the genetic variability and the level of genetic control of disease resistance based on progeny testing. The results of these activities will help support the development of a more resistant population of F. excelsior for planting in Swedish forests, cities, and landscapes.

Spatial distribution of fungal communities in the stems of healthy and declining *Populus* species commonly used for bioenergy resource in Southern Sweden: Implications for productivity

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Growing poplars has become increasingly popular on both agriculture and forest land in southern Sweden. Productivity of biomass is expected to be high but may be compromised by infection by various fungal root and stem diseases. To study fungal communities and potential effect on the growth, 22 stands of hybrid aspen (*P. tremula* \times P. tremuloides) and hybrid poplar (P. maximowiczii × P. trichocarpa) were inventoried, including some with clear stem damage and declining health status (visible loss in percent live crown). In each stand, trees were measured and damage and vitality classes were recorded. Within each stand, 20 trees were selected for more extensive sampling. Wood samples from the stem (including both heartwood and sapwood) were taken at stump height, 3-3.7 m height on the stem, and from visible wounds (stem cankers). On wood cores, discoloration and decay were noted and then samples were immediately frozen for further processing in the lab. In addition, discs were collected from felled trees and the extent of decay in the stems were assessed by cutting the stem in 0.5 m sections. Additional sections were collected from the zone of decay, transition zone and sapwood/ cambial zone. In the lab, wood samples were ground to fine powder, and then DNA was extracted and amplified with PCR using fungal specific ITS1/4 primers. Barcoded samples were sequenced on a PacBio RS II sequencer. Results presented will describe the fungal communities associated with healthy and declining Populus species including the spatial distribution of those communities within the tree.

Mycorrhization for improvement conifer seedlings quality in Montenegro

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Successful afforestation, plant survival and fast development of seedlings depend on their quality. It is of special importance for South-eastern European region, due to unfavourable environmental conditions: prevailing high summer temperatures of air and soil, low humidity and low precipitation during growing season. Nursery production in Submediterranean and Mediterranean region is facing the same problems.

Seedling mycorrhization is important for achieving higher seedlings' quality. Mycorrhiza provides protection of seedlings against pathogens. It improves water uptake, mineral nutrition, growth and adaptation of forest trees as well.

Possibilities for involvement of different techniques for ectomycorrhizal inoculation of containerized *Pinus nigra* seedlings in nursery production in open field were studied. Ten native ectomycorrhizal fungi were examined as vegetative and spore inoculums, also as the soils from four unmanaged forests. Effect of inoculations on ectomycorrizal formation and seedling growth characteristics were tested.

The utilisation of spore (10⁶, 10⁷, 10⁸) and vegetative (1:4, 1:8, 1:16) inoculums of *Pisolithus arhizus* and *Suillus granulatus* proved to be an effective method for obtaining containerized ectomycorrhizal *P. nigra* seedlings in open field conditions. According to ectomycorrhizal development on seedlings roots, even better effect were achieved after first growing season by using of unmanaged forests' soils from *Abieti-Fagenion moesiacae* and from *Pinetum heldreichii* forests as ectomycorrhizal inoculums (1:9; 1:19).

Controlled mycorrhizal inoculation of seedlings is not a common practice in Montenegrin nurseries. A new technology which considers mycorrhization, including alteration in growing substrates, regimes of fertilization and pesticides' applications different than those commonly used in Montenegrin nurseries, could be adopted for achieving better seedling quality of *Pinus nigra* and the other conifers.

Occurrence of *Dothistroma septosporum* on high altitude pine forests in Montenegro

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Pinus heldreichii and *P. peuce* are high-elevation pines of the Mediterreanean basin. They inhabits a forest zone on the upper forest line in high mountain regions in the western part of Balkan peninsula, influenced by the Mediterranean climate, on altitudes between 1200-2000 m, where they fulfil an important ecological role. Being tertiary relicts and endemics, they have limited area of natural distribution and, hence, require a special attention and implementation of measures for its conservation. The presence of Dothistroma needle blight was investigated in these forests in Montenegro.

This is the first time *D. septosporum* was detected from native forests of *P. heldreichii* and *P. peuce* and the first report of DNB on these two pine species in Montenegro. On *P. heldreichii*, *D. septosporum* was detected in three different mountain massifs (Žijevo, Prekornica and Prokletije). At two localities *D. septosporum* was identified by species specific primers, and on the third site DNB was identified morphologically. Presence of *D. septosporum* was also confirmed with species specific primers from *P. peuce* growing in a primeval native forest at Mt. Prokletije, while no characteristic symptoms were observed.

The intensity of the disease was low both in *P. heldreichii* and *P. peuce* forests probably due to the prevailing environmental conditions which are not favorable for DNB development.

Using molecular methods (conventional PCR with species specific primers), *D. septosporum* was also detected in needles of *P. nigra*, *P. sylvestris* and *P. halepensis*, while *D. pini* was confirmed only in one needle sample from *P. sylvestris* in Montenegro.

Gene expression in European Ash in response to inoculation with Hymenoscyphus fraxineus

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European Ash is threatened by the invasive pathogen *Hymenoscyphus fraxineus*, causing Ash dieback disease. The objective of this study is to profile host defense responses using high-throughput cDNA sequencing (RNA-seq) to gain an understanding of key processes.

To meet this objective high-throughput cDNA sequencing (RNA-seq) data generated by Illumina sequencing has been obtained from Ash bark subjected to different experimental treatments. The RNA-seq data are analyzed by TopHat and Cufflinks.

In a preliminary study 204 transcripts were differentially expressed in inoculated bark compared to control and 151 transcripts in the comparison between wounding and inoculation. Several of the differentially expressed genes belonged to the abscisic acid (ABA) signaling pathway. The role of ABA signaling in lesion development will be discussed.

Fungi associated to necrotic galls of *Dryocosmos kuriphilus* (Hymenoptera; Cynipidae) in north Spain: preliminary results

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The invasive chestnut gall wasp *Dryocosmos kuriphilus* is one of the most important pests on *Castanea* species all over the world (EPPO, 2005) with nut losses reaching the 80 % when severe infestations. In 2012, the Asian gall wasp (AGW) was found for the first time in Cataluña (Spain) and one year later, we detected its presence in Cantabria, north Spain.

Several authors have previously found the relationship between the presence of some *Fusarium* species in the necrotic galls and wasp morality, due to the production of entomopathogenic toxins like fumonisin or (+)-ABA.

The aim of this study is to investigate this relationship in order to find interesting perspectives for the AGW biocontrol. For this purpose, 181 necrotic galls from two *Castanea sativa* trees were collected from June to September 2014 in Vejorís (Cantabria). One hundred and twenty galls were processed and cultured with the aim of isolating and identifying associated fungi. Fungal isolates were classified into morphotypes attending to macromorphological features and selected for DNA amplification and sequencing, with special attention to *Fusarium*-like isolates.

A total of 8 fungal species were identified: *Fusarium* sp.; *Fusarium avenaceum*, *Fusarium oxysporum*, *Pestalotiopsis* sp., *Epicoccum* sp., *Epicoccum nigrum*, *Penicillium ra-mulosum* and *Gnomoniopsis smithogilvyi*. Deeper studies are required in order to verify the possible parasitic effect of *Fusarium* species as biocontrol agents against the AGW, due to their capacity of direct penetration in the galls, leaves and twigs, highlighting the importance of *F. oxysporum* as an entomopathogenic fungus.

Keywords: Asian gall wasp, Castanea sativa, Fusarium, entomopathogens.

Phytophthora spp. in nurseries and gardening centres in the Czech Republic

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Phytophthora spp. pathogens belong among the most dangerous pathogens of woody plants in the world. Many of these pathogens can spread with infected plants from nurseries and gardening centres. The presented review summarizes the findings of *Phytophthora* pathogens deposited in the Czech Collection of Phytopathogenic Oomycetes from nurseries and gardening centres in the Czech Republic.

There were identified more than 30 species of pathogenic oomycetes including 18 *Phytophthora* taxa during 2007 – 2014. The most frequently acquired *Phytophthora* pathogens were *Phytophthora plurivora*, *P. cinnamomi*, *P. cactorum*, *P. cambivora* and *P. citrophthora*. These pathogens were often isolated from *Ericaceae* and other ornamental plants.

The emphasis was put also on pathogens of seedling of native woody plants in forest nurseries. The most important acquired pathogens from these hosts were *P. plurivora*, *P. cambivora*, *Phytophthora alni* and *P. cryptogea* isolated from *Alnus glutinosa*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus robur*, *Tilia cordata* and other species.

The trade with ornamental- and forest woody plants is important pathway of spread of *Phytophthora* pathogens in the area. The use of seedlings from infected nurseries poses the high risk for health stage of Czech forests.

Gemmamyces piceae – a new important threat for Colorado blue spruce cultivation

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Gemmamyces piceae (syn. *Cucurbitaria piceae*), the cause of bud blight of *Picea pungens* Engelm. and some other coniferous woody plants was firstly described about one hundred years ago in Europe. The pathogen was reported as a rather unimportant pathogen causing local losses at the most. The situation dramatically changed recently, when many substitute blue spruce stands were heavily damaged or killed by epidemy of gemmamyces bud blight in Northern Bohemia. The affected blue spruce plantations were established in mountain areas deforested in the consequence of sulphur dioxide depositions during the second half of 20th century.

The damage develops during spring, when small brownish lesions form in germinating buds (the pathogen was consistently isolated from them). The affected buds proliferate unequally, twist and later die. High amount of blackish fructification bodies of imperfect stage cover the surface of colonised buds during summer; the perfect stage develops later in season.

The pathogen usually kills high proportion of buds of affected trees (more than 75 % in highly affected stands), thus number of newly sprouting shoots is highly limited and defoliation quickly increases as older classes of needles fall off. The highly affected trees die during a few years.

Hundreds of hectares of trees were highly affected or killed by the pathogen especially in the Krušné hory Mts. The situation is also serious in other North Bohemian mountains with blue spruce plantations and, moreover, the pathogen spreads across the country in lower altitudes. The pathogen poses an important threat also for ornamental stands, nurseries and plantations of Christmas trees. The pathogen is apparently capable of causing enormous losses in the case of its introduction into the native area of its host.

Fungi associated with shoot dieback of birch (*Betula pendula* Roth.) seedlings in nursery and after outplanting in forest: preliminary results

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Dieback of birch shoots was observed in some Latvian forest nurseries, as well as poor survival of planted seedlings in forest stands was reported. The aim of this study was to find out the causal agent of birch dieback. In total, 68 symptomatic birch seedlings were analysed (23 from forest nursery "Zābaki" and 45 – recently (1-2 years ago) outplanted in forest stands). For all seedlings shoots necroses and bark damaged were evaluated. From each shoot wood and bark samples were taken for fungal isolation. For fungal identification morphological and molecular methods were used. As a result, 24 different fungal species were isolated. The most common fungus detected was *Melanconis stilbostoma* (*Melanconium bicolour*). This year more samples will be collected in 4 biggest forest nurseries of Latvia (at least 50 seedlings from each).

The European race of *Gremmeniella abietina* hosts a single species of Gammapartitivirus showing a global distribution and possible recombinant events in its history

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The population genetics of the family *Partitiviridae* was studied within the European race of the conifer pathogen Gremmeniella abietina. One hundred sixty-two isolates were collected from different countries, including Canada, the Czech Republic, Finland, Italy, Montenegro, Serbia, Spain, Switzerland, Turkey and the United States. A unique species of Gremmeniella abietina RNA virus-MS1 (GaRV-MS1) appears to occur indistinctly in G. abietina biotypes A and B, without a particular geographical distribution pattern. Forty-six isolates were shown to host GaRV-MS1 according to direct specific RT-PCR screening, and the virus was more common in biotype A than B. Phylogenetic analysis based on 46 partial coat protein (CP) cDNA sequences divided the GaRV-MS1 population into two closely related clades, while RNA-dependent RNA polymerase (RdRp) sequences revealed only one clade. The evolution of the virus appears to mainly occur through purifying selection but also through recombination. Recombination events were detected within alignments of the three complete CP and RdRp sequences of GaRV-MS1. This is the first time that recombination events have been directly identified in fungal partitiviruses and in G. abieting in particular. The results suggest that the population dynamics of GaRV-MS1 do not have a direct impact on the genetic structure of its host, G. abietina, though they might have had an innocuous ancestral relationship.

Occurrence and implications of putative mycoviral dsRNA molecules in the ash dieback causal agent, *Hymenoscyphus fraxineus*

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As a primary indicator of putative mycovirus presence, the occurrence of double stranded (ds) RNA molecules was investigated within the ash dieback causal agent, *Hymenoscyphus fraxineus*. A total of 154 isolates from nine different European countries (Austria, Belgium, Czech Republic, Norway, Latvia, Lithuania, Poland, Russia and Slovakia) were screened, and three different dsRNA bands of *ca* 2.2, 2.5 kb and 5 kb were confirmed to occur in almost 28 % of the examined *H. fraxineus* isolates. The most frequent band had a size of *ca* 2.2. kb, and seems to correspond to *Hymenoscyphus fraxineus* mitovirus 1 (HfMV1). To determine if the dsRNA occurrence had any effect on the morphology of *H. fraxineus*, an in vitro experiment was carried out. Thus, despite observing mycelium debilitation in some mycovirus-hosting cultures, statistical analyses did not support a significant relation between the dsRNA presence and the growth rate and colour of the mycelium. Nevertheless, a significant correlation was found between the mycelial growth rate and colour. The darker mycelia appeared to grow slower. This study is the first step for the detection of mycoviruses in *H. fraxineus* and sets up the bases for further biocontrol examinations.

Throwing the baby out with the bath water? - Any interactions between foliar pathogens and mycorrhizal fungi in Norway spruce seedlings?

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We studied whether the induction of defence against foliar pathogens affects the interaction of Norway spruce with ectomycorrhizal fungi (EMF) and whether the response differs between seedlings originating from families showing variable growth performance in long-term trials. The shoots were inoculated with Botrytis cinerea and Gibberella avenacea. The roots were simultaneously inoculated with sieved humus to provide the EMF inoculum. The severity of the pathogenic infection was based on the amount of induced production of condensed tannins and damage in the needles. EMF richness and colonisation were not affected by the pathogens and were also identical between the fast- and slow-growing seedlings. The fast-growing seedlings were more vulnerable to the pathogens; however, constitutive level of condensed tannins in the needles did not correlate with their susceptibility to either the pathogens or ECM fungi. G. avenacea induced a marginally greater production of condensed tannins in the slow-growing seedlings, which was linked to slight reduction in EMF richness and less needle damage after wintering. The results suggest that there are differences in resource allocation strategies between the fast- and slow-growing families, which may indicate presence of underlying host effects that regulate interactions with associated fungi. Vice versa, we also tested if root colonization by EMF Meliniomyces sp., which has shown antagonistic properties towards Heterobasidion sp. in vitro, could protect seedlings against foliar B. cinerea or Gremmeniella abietina infections. Seedlings inoculated with Meliniomyces showed transiently less G. abietina-induced needle damages during the first growing season, but the effect disappeared during the next season.

Virulence of *Hymenoscyphus fraxineus* isolates from Lithuanian (post-epidemic) and Swiss (epidemic) populations

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A severe ash dieback caused by an invasive ascomycete *Hymenoscyphus fraxineus* is currently observed in most European countries. The main aim of this study was to determine if there are significant differences in virulence between *H. fraxineus* isolates originating from two geographically distant populations: epidemic (Swiss) and post-epidemic (Lithuanian), and between isolates representing two phases of pathogen's life cycle - parasitic (recovered from necrotic lesions on living trees) and saprophytic (from fallen leaf petioles). In September 2013, an inoculation experiment was conducted on 3-year-old *Fraxinus excelsior* seedlings (N=1,000) using 100 *H. fraxineus* isolates from each country, of which 50% were lesion and 50% petiole isolates. Five seedlings were inoculated with each isolate.

In June 2014, bark necroses induced by Lithuanian isolates (mean length measured downwards the inoculation point = 6.02 ± 0.26 cm) were slightly larger (non-significant difference, p=0.343) than those induced by the Swiss ones (5.65 ± 0.29 cm). However, significant differences (at p=0.004) were observed between mean downward length of necroses induced by lesion and petiole isolates: 4.58 ± 0.25 cm vs. 5.68 ± 0.24 cm, respectively. Our results didn't support the hypothesis that higher virulence of a pathogen may be selected in an expanding epidemic than in a declining (post-epidemic) one, although there is a strong indication of existence of epigenetic differences or niche partitioning between *H. fraxineus* individuals representing different phases (parasitic vs. saprophytic) of the pathogen's life cycle. Moreover, fitness trade-offs could occur between within-season growth (loss of fitness due to a continuous interaction between the fungus and a self-defending host plant) and off-season survival (the "resting" saprophytic phase).

Occurrence and genetic diversity of *Phytophthora alni* s.l. in Lithuania and Switzerland

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Phytophthora alni sensu lato is a recently emerged hybrid pathogen that causes lethal root and collar rot in alders (*Alnus* spp.) The disease was first reported in 1993 in England and is currently widespread across Europe, including Lithuania and Switzerland. In Lithuania *P. alni* s.l. was for the first time isolated and identified from declining alder trees in 1999 and in Switzerland in 2006. However, no detailed studies have been conducted on significance and distribution of the alder decline in both countries. Moreover, no information is available about which of the three subspecies of the *P. alni* complex (*P. alni* ssp. *alni*, *P. alni* ssp. *multiformis* or *P. alni* ssp. *uniformis*) is/are present. Similarly, amount and distribution of genetic diversity within the occurring subspecies is still unknown.

In this study we aimed: (1) To determine the distribution and incidence of the alder decline in Switzerland and Lithuania; (2) To determine which species of the *P. alni* complex is/are responsible for the observed decline; And (3) to characterize the genetic structure of the sampled *P. alni s.l.* populations using microsatellite markers.

Associated bacteria of *Agrilus biguttatus* sampled from English oak displaying symptoms of acute oak decline

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Acute oak decline is a syndrome affecting native oak trees in Britain. The Buprestid, Agrilus biguttatus is consistently associated with symptomatic trees. Concurrently, specific bacteria are isolated from symptomatic trees throughout. To understand potential transmission and transport of microorganisms by this insect, more knowledge is required on the presence of internally and externally occurring prokaryotes. A previous study identified inter alia members of the Alpha- and Gammaproteobacteria as well as Firmicutes including plant pathogenic species in the gut of Agrilus planipennis Fairmaire, the emerald ash borer (Vasanthakumar et al., 2008) highlighting the potential of the beetle to act as important vector. Freshly emerged adults of both sexes and neonate larvae were studied. Specifically, bark from symptomatic trees infested with A. biguttatus larvae was collected from three woodlands in England from winter 2013 to spring 2014 and placed in separate emergence cages. After emergence in summer 2014, beetles were housed separately by sampling site in the laboratory, fed non-symptomatic oak leaves, and allowed to mate and oviposit. Eggs were incubated at 22.5°C until hatching. Genomic DNA of bacteria associated with the beetles or larvae was extracted from the samples using mechanical lysis (MoBio[®] PowerSoil Kit). The samples underwent microbial community profiling using fragments of the 16S rRNA gene followed by pyrosequencing. To our knowledge this is the first report of bacterial association with Agrilus biguttatus using cultivation independent methods.

Vasanthakumar et al., 2008. Gut microbiota of an invasive subcortical beetle, Agrilus planipennis Fairmaire, across various life stages. Environ Entomol. 37(5):1344-53.

Molecular identification of pine tree-infecting phytoplasmas from the UNESCOprotected Curonian Spit in Lithuania

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Although phytoplasmas are mainly known as pathogens that affect angiosperms, phytoplasmas have recently been detected in diseased coniferous plants. In 2011-2014, we observed, in the Curonian Spit of western Lithuania, diseased trees of Scots pine (Pinus sylvestris) and mountain pine (Pinus mugo) with unusual symptoms similar to those caused by phytoplasmas. Diseased trees exhibited excessive branching, dwarfed red or yellow needles, dried shoots and ball-like structures. Scots pine is a native tree in Europe and Asia, and Mountain pine (native to Alpine mountains) was introduced from Denmark. The existence of phytoplasmal disease of pines could pose the threat of losses in the wood industry and damage to the Spit dunes ecosystem. RFLP and nucleotide sequence analysis of 16S rRNA gene fragments revealed that individual trees were infected by 'Candidatus Phytoplasma pini'-related strains (members of phytoplasma subgroup 16SrXXI-A) or by 'Ca. Phytoplasma asteris'-related strains (subgroup 16SrI-A). Of the nearly 300 trees that were sampled, 80% were infected by 'Ca. Phytoplasma pini'-related strains. Strains belonging to subgroup 16SrI-A were identified from only few trees. Use of an additional molecular marker, secA, supported the findings. Further work will be needed to identify the currently unknown insect vector(s) that are responsible for spread of these phytoplasmas among pine trees in the country. This study provides evidence of large scale infection of *Pinus* by '*Ca*. Phytoplasma pini' in Lithuania, and it reveals that this phytoplasma is more widespread geographically than previously appreciated.

The effect of copper and silver nanoparticles on growth and mycorrhizal colonization of Scots pine (*Pinus sylvestris* L.) and pedunculate oak (*Quercus robur* L.) seedlings in a nursery experiment

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Some studies indicate that the metal nanoparticles (NPs) can be used in plant cultivation as fungicides and growth promoters. The aim of the study was to evaluate the effect of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) on the growth and condition of mycorrhizae of one-year old seedlings of Scots pine (Pinus sylvestris L.) and English oak (Ouercus robur L.) growing in containers. Nanoparticles were applied to foliage four times, at three concentrations of 5, 25 and 50 ppm. None of the concentration of AgNPs and CuNPs had any significant effect on growth parameters of oak. All concentrations of AgNPs and CuNPs reduced root dry mass of pine seedlings. Additionally pine seedlings treated with AgNPs were characterized by smaller: shoots length, root dry mass and root diameter. However the lowest concentration of CuNPs stimulated the growth of shoot significantly. Four mycorrhizal taxa were detected on the pine (Sphaerosporella brunnea, Thelephora terrestris, Suillus bovinus, Peziza sp.) and oak roots (S. brunnea, T. terrestris, Paxillus involutus, Laccaria proxima). No quantitative or qualitative differences in the level of colonization of oak roots depending on the NPs type and concentration were detected. In pines treatment with NPs at concentrations of 5 and 25 ppm significantly raised the general mycorrhization level. Our studies indicate a differential impact of NPs on the growth of seedlings of forest trees, depending on the species, type of NPs and their concentration and the ability to stimulate the formation of mycorrhizae by NPs.

Species composition of an ectomycorrhizal fungal community along a local nutrient gradient and soil texture in declining oaks (*Quercus robur* L.) stands in the Krotoszyn Plateau

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The aim of the present study was (i) to relate the composition of ectomycorrhizal (EcM) species and abundance of vital mycorrhizal root tips (VM) to geologically different sites, (ii) to show how variable site conditions contribute to EcM community changes and (iii) to show the occurrence of non-mycorrhizal (NM) and non-vital (NV) root tips in declining oak stands. For this, we describe the (EcM) root tips and their diversity of mycorrhizal fungal species on three *Quercus robur* sites (120 years old and 60 years old respectively). Soil properties, mycorrhizal fungi abundance and vitality of stands (crown transparency, vitality root parameters etc.) were assessed. The declining oak stands were characterized by a low degree of mycorrhization: 28,8%, 27,9% and 24,8% at Piaski (P), Krotoszyn (K) and Karczma Borowa (KB) sites, respectively. On the latter stand (KB) we observed also the largest amount of NV root tips (70,4%), followed by K (57,1%)and P (48,1%). By morphotyping and direct sequencing of root tips from 180 trees we detected a total of 21 taxa of EcM fungi: 12 at K and 15 both at KB and P sites. The nonparametric Spearman-correlation analysis showed significant correlations of soil and roots parameters with NV and NM root tips. NV root tips were negatively related to organic carbon (C_{orr}) (r=-0,16), however, positive correlation was found to Mg content. The strongest positive correlations showed NM root tips to C_{orr}, total N, C/N ratio, whereas NM were negatively related to pH(r=-0,2).

Ash dieback in Bavaria- *Hymenoscyphus fraxineus* in combination with secondary pathogens

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Since its first identification in Poland in 2006, the ascomycete *Hymenoscyphus fraxineus* has caused massive dieback of *Fraxinus excelsior* in countries of eastern, northern and central Europe. Typical disease symptoms are the wilting of the leaves, necrotic lesions in the bark and reddish discolorations of stems and branches. This work shows the development of disease expansion and severity in Bavaria for a period of five years, starting in 2010. Special study attention is turned to secondary pathogens, like *Hylesinus fraxinus* and species of *Armillaria*. It is shown that breeding galleries of *Hylesinus fraxini* are only found in recently died back trees still acting as secondary opportunistic pathogens. In contrast, *Armillaria spp*. can be considered as serious pathogens of weakened ash trees. In different ash stands typical symptoms of infection can be found. A relationship between stem base necrotic lesions and vitality was also examined. It is shown that these fungi contribute severely to an accelerating mortality in weakened ash stands. In the following years, forest conversion will have to be urgently considered to avoid tree deficiency on large areas.

Effect of chitosan on disease control and growth of Scots pine (*Pinus sylvestris*) in the forest nursery

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Chitosan has become a promising biological agent for disease control and plant growth promotion. The objective of this study was to assess the effects of chitosan, applied as an active ingredient of Beta-chikol (Poli-Farm, Łowicz, Poland), to control dampingoff and Lophodermium needle cast on Scots pine seedlings growing in field conditions. Beta-chikol was used for seed treatment and as foliar spray at recommended rates and concentrations. Seedlings protected with fungicides and unprotected ones were used for comparison. For each experimental variant (chitosan, fungicides, unprotected) after germination and 6 weeks later, inventories of seedlings were performed and all individuals were counted on 32 segments (1 m long each) of seed rows. In the aboveground parts of seedlings, the concentration of endogenous salicylic acid was determined by HPLC. At the end of the growing season, seedling growth parameters were determined. Beta-chikol used as foliar spray limited infection by damping-off fungi, but was ineffective when used as seed treatment. For this reason, a better protective effect was achieved when fungicides were applied. Lophodermium needle cast was not observed during the study period. After the application of Beta-chikol, the concentration of salicylic acid did not increase. Moreover, its higher level was detected in the case of unprotected seedlings. This finding could be a result of plant defence response to damping-off infection. The application of Beta-chikol enhanced all growth parameters under investigation. Our results indicate a possibility to use chitosan in the form of Beta-chikol to stimulate plant growth and protect pine seedlings against parasitic damping-off in forest nurseries.

The cork forests in the region of Edough (Algeria) are they in danger?

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The majority of Algerian cork oak forests shows a serious state of decay which leads to a reduction in their area. The cork oak forests of Eastern Algeria do escape this situation. We set up in 2010 a permanent observation station (El Berouaga) in the Edough forest to study the health status and different decline factors of cork oak. We conducted dendrometric surveys (girth taken at 1.30 m from the ground, tree height and height of first branches), and cork statements (height and number of debarking, debarking coefficient) on 80 trees. During the fall of 2010 we evaluated the health status of the stands by examining crown and trunk, bark and subcortical area. Trees have on average 8 m high, 1.60 m in circumference, and 1.80 m in debarking heigth. Over 57% have a low defoliation and 45% low leaf discoloration; calculated decay index indicates a stand at the beginning of decline (Id = 1.70). The lichenous flora is present on all the trunks. Few trees have carbonaceous crusts, runny or dehiscent cork surfaces while insect holes are visible over 60% of the sampled trees. Human impact remains limited despite 25% of the trees present injuries.

Keywords: Biodiversity, cork oak, decay, Edough, Algeria.

Investigation of fungicide resistance in *Botrytis cinerea* in forest nurseries in Norway

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Norway spruce (*Picea abies*) seedlings in forest nurseries are routinely treated with fungicides to control grey mould caused by *Botrytis cinerea*. Fungicides in three chemical groups are registered for use to control grey mould: the dicarboximide iprodione, the benzimidazole thiofanate-methyl, and the hydroxyanilide fenhexamid. Chemicals from the two first groups have been used for several decades, and reports on resistance development in *B. cinerea* to these groups were published few years after their release. Fenhexamid has been on the market since 2001, and reports from many countries show that resistance has developed in strains of *B. cinerea* in many vegetable and fruit crops. Due to heavy grey mould infestations in spruce seedlings in recent years despite regular fungicide applications, a project to investigate the presence and frequency of resistant *B. cinerea* strains in forest nurseries was granted (2014-2016). Preliminary results showed that there were strains resistant to fenhexamid in three out of six nurseries. Multiresistant strains were found in one out of three nurseries. Frequency of resistant strains in Norwegian forest nurseries are currently investigated.

Fungal community of wind and salt exposed Scots pine needles

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Tree foliage is a habitat for diverse fungal communities including endophytic and pathogenic fungi. Trees growing in urban areas are often subjected to different abiotic stressors. Roadsides may be an especially stressful environment with exposure to traffic, wind and, in colder regions, also highway salt used to keep roads and highways safe during adverse winter weather conditions. Exposure to wind and salt may pose a stress to the trees, increase the susceptibility of infection by pathogens and change the fungal community structure of foliar fungi. The main aim of this project was to analyse shifts in the community of pathogenic and endophytic fungi of Scots pine needles in relation to abiotic stress and to identify species responding to salt and wind exposure by increasing or decreasing their abundances. In 2010, Pinus sylvestris needles were sampled along European highway (E4) between Stockholm and Uppsala, Sweden, and in locations in the vicinity to include sites with and without salt exposure and wind. Different yearly cohorts and needles with and without symptoms were sampled separately. The fungal community composition was analysed using 454-pyrosequencing. The fungal community was dominated by well-known pine needle species such as different Lophodermium species and Sydowia polyspora. Preliminary analysis showed that salt exposure, but not wind, appear to alter the foliar fungal community composition. The most common species, the known pathogen Lophodermium seditiosum, also increased in abundance in needles exposed to salt.

Molecular based detection of Dothistroma septosporum conidia by air sampling

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Our target is a description of *Dothistroma septosporum* (causal agent of Dothistroma needle blight) spore dispersal in order to examine its biological relation to climatic conditions in the Czech Republic and to optimize the technology of its detection by real-time PCR. For this experiment one infected locality with infected plant material of few species of pines in the Czech Republic was chosen. In the locality an automatic volumetric spore trap and climatic station were installed to continuously sample the aerospora. In few cases, a rotating arm spore trap was also used to obtain 48-hour air samples and to compare the efficiency of the two types of spore traps. To cover the known period of *D. septosporum* spore release, the air sampling lasted from the beginning of April to the beginning of November 2014. The samples were divided into sections per days, from which the *D. septosporum* DNA was extracted and quantified by specific real-time PCR with newly designed primers and dual-labeled probe. First results show a higher efficiency of the rotating arm type spore trap, which might be caused by the higher volume of air sampled.

Evolutionary history of the pine needle pathogen Lecanosticta acicola

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Lecanosticta acicola (sexual state: Mycosphaerella dearnessii) is a heterothallic ascomycete that causes brown spot needle blight (BSNB) on pines. Severe infections can lead to retardation of growth and death of pines. L. acicola has a global distribution and infects both native and non-native Pinus spp., particularly in North America and Europe. The objective of this study was to consider the origin of European populations of L. acicola. Part of the Translation Elongation Factor 1- α (EF) gene was sequenced in order to determine phylogenetic relationships among different EF haplotypes. For the population genetic analyses, eleven microsatellite loci were screened on 201 L. acicola isolates from 29 locations (19 countries). Phylogenetic analyses indicated that the Central American haplotypes of L. acicola are highly diverse and ancestral to those found elsewhere in the world. Microsatellite data analyses distinguished two distinct populations in North America: northern and southern lineage. Approximate Bayesian Computation (ABC) analyses supported independent introductions of these lineages into Europe. Interestingly, these two European clusters of L. acicola reflect the same geographic distribution as that of the North-American populations which is probably related to climatic and/or host adaption.

Diplodia sapinea found on Picea spp. in Norway and Denmark

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Diplodia sapinea is mainly found on pine (*Pinus* spp.) worldwide, but has a wide range of conifer host plants, including spruce (*Picea* spp.). The fungus was found in Norway on common juniper (*Juniperus communis*) in 2002 and on Norway spruce (*Picea abies*) in forest stands in Vestfold County in 2013 and in Akershus County in 2015. In Denmark, there are a few recent records of *D. sapinea* from Scots pine (*Pinus sylvestris*) on Sealand and Møn in 2007 and 2012, but the earliest reported find was on mountain pine (*P. mugo*) in Jutland in 1899. In 2014, the fungus was found on dead shoots of young Serbian spruce (*P. omorika*) planted under Scots pine and lodgepole pine (*P. contorta*) shelter trees in North Jutland. *D. sapinea* was identified based on morphology of fruitbodies (pycnidia) and spores (conidia). Sequencing will be performed to confirm identification. Prior to the recent findings of *D. sapinea* on spruce in Norway and Denmark, the fungus has never been reported on spruce in Scandinavia. An isolate of the fungus from Serbian spruce proved to be pathogenic to Norway spruce in an inoculation experiment.

Diplodia shoot blight: Impact, risk and forest species response

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Diplodia pinea causes shoot blight and branch cankers mainly in *Pinus spp*. This species that cause Diplodia blight can live in a host tree for years without causing disease, remaining inactive until the plant suffers weakness or stress-hail. 24 % of sampled plantations showed damages associated to Diplodia blight compared to around 98 % of sampled plantations in which presence of the species was confirmed.

Damages caused by *D. pinea* have been associated with wounds made by hail storms, insects or management activities like pruning or shearing operations. Wounded tissues remain vulnerable to *D. pinea* infection for several days. In the Basque Country *D. pinea* has often severely damaged trees wounded by hail. In addition rainfall and radiation are also defined as predisposal factors to spatial risk of disease development. Predicted probability of disease incidence was estimated around 20 % of the studied area.

The susceptibility levels against Diplodia shoot blight were evaluated for *Pinus* species present in the area by performing 2 experiments in greenhouse conditions. Remarkable differences among average values of length lesions in inoculated seedlings were detected being *P. radiata* the most susceptible, with lesion length ranging from 1.2 to 32.5 mm. In addition, some individual of other conifer species considered resistant are also remarkably susceptible. The inter and intra specific susceptibility differences observed together with the spatial analysis of disease risk will constitute the key to define strategies of control and prevention of damages caused by this species.

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Poster Communications

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Woodward, S.	F. Alenezi, S. Fraser, Bełka, M. Doğmuş, T.H., Heckova, Z., Oskay, F. Woodward, S.	Aneurinibacillus migulanus Nagano controls Dothistroma Needle Blight of Pine	s.woodward@abdn.ac.uk
E. Iturritxa	E. Ortiz de Urbina, R. Raposo, M. Elvira-Recuenco, A. Aragonés, N. Mesanza, Ch. Pattenc, J. Aitkend, E. Iturritxa	Outbreak of needle blight diseases in Pinus radiata: emerg- ing forest diseases in the North of Spain	eiturritxa@neiker.net
Michelle Cleary	Michelle Cleary, Lars-Göran Stener, Jan Stenlid	Selection and testing Fraxinus excelsior genotypes for resist- ance against Hyme- noscyphus fraxineus in Sweden	Michelle.Cleary@slu.se
Michelle Cleary	Gunulf-Åberg, A. Cleary, M. Rönnberg, J.	Spatial distribution of fungal communi- ties in the stems of healthy and declining Populus species commonly used for bioenergy resource in Southern Sweden: Implications for productivity	Michelle.Cleary@slu.se
Lazarevic, J.	Lazarević, J Keča, N.	Mycorrhization for improvement conifer seedlings quality in Montenegro	ena.lazarevic@gmail.com
Lazarevic, J.	Lazarević J. Davidenko K. Milberg, H.	Occurrence of Dothistroma sep- tosporum on high altitude pine forests in Montenegro	ena.lazarevic@gmail.com
Eshghi-Sahraei,S.	Eshghi-Sahraei, S. Cleary, M. Brandström Durling, M. Stenlid, J. Elfstrand M	Gene expression in European Ash in re- sponse to inoculation with Hymenoscyphus fraxineus	shei0001@stud.slu.se
Fernández M.M.	Bezos, D., Diez, J.J. Fernández, M.M.	Fungi associated to necrotic galls of Dryocosmos kuriphi- lus (Hymenoptera; Cynipidae) in north Spain: preliminary results	mffernan@agro.uva.es
Mrázková, M.	Mrázková, M. Hrabětová M., L. Černý, K.	Phytophthora spp. in nurseries and gar- dening centres in the Czech Republic	mrazkova@vukoz.cz

Černý, K.	Černý K., Hrabětová M., Soukup F., Pešková V.	Gemmamyces piceae – a new important threat for Colorado blue spruce cultivation	cerny@vukoz.cz
Gaitnieks, T.	Gaitnieks, T., Arhipova, N., Liepiņš, K.	Fungi associated with shoot dieback of birch (Betula pendula Roth.) seedlings in nursery and after outplanting in forest: preliminary results	talis.gaitnieks@silava.lv
Leticia Botella	Leticia Botella, Tero T. Tuomivirta, Jarkko Hantula, Julio J. Diez, Libor Jankovsky	The European race of Gremmeniella abietina hosts a single species of Gammapartitivirus showing a global dis- tribution and possible recombinant events in its history	leticia.sanchez@mendelu.cz
Vendula Čer- máková	Vendula Čermáková, Libor Jankovský, Leticia Botella	Occurrence and im- plications of putative mycoviral dsRNA molecules in the ash dieback causal agent, Hymenoscyphus fraxineus	vendula.cermakova@mendelu.cz
Pennanen, T.	Velmala, S.M., Rajala, T., Smolander, A., Vuorinen, I., Lilja, A., Uimari, A. Pennanen, T.	Throwing the baby out with the bath water? - Any interactions between foliar pathogens and mycorrhizal fungi in Norway spruce seedlings?	taina.pennanen@luke.fi
Lygis, V.	Lygis, V. Rigling, D. Burokienė, D. Marčiulynienė, D. Schoebel, C. N. Norkutė, G.	Virulence of Hyme- noscyphus fraxineus isolates from Lithua- nian (post-epidemic) and Swiss (epidemic) populations	diana.marciulyniene@gmail.com
Norkute, G.	Norkute, G. Lygis, V. Prospero, S.	Occurrence and genetic diversity of Phytophthora alni s.l. in Lithuania and Switzerland	goda.norkute@wsl.ch
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Valiunas, D.	Valiunas, D. Jomantiene, R., Iva- nauskas, A., Urbonaite, I., Davis, R.E.	Molecular iden- tification of pine tree-infecting phy- toplasmas from the UNESCO-protected Curonian Spit in Lithuania	deividas.valiunas@gmail.com
Olchowik, J.	Olchowik, J., Szaniawski A., Bzdyk R., Aleksandrowicz – Trzcińska M., Urban A.	The effect of copper and silver nanopar- ticles on growth and mycorrhizal coloniza- tion of Scots pine (Pinus sylvestris L.) and pedunculate oak (Quercus robur L.) seedlings in a nursery experiment	olchowik.jacek@gmail.com
Bzdyk, R.	Bzdyk R., Kohler J., Tkaczyk M., Kubiak K.A., Nowakowska J.A., Oszako T.	Species composition of an ectomycorrhizal fungal community along a local nutrient gradient and soil texture in declining oaks (Quercus robur L.) stands in the Krotoszyn Plateau	romanbzdyk@gmail.com
Lenz, H.D.	Lenz, H.D., Bartha, B., Straßer, L.	Ash dieback in Bavaria- Hymenos- cyphus fraxineus in combination with secondary pathogens	heike.lenz@lwf.bayern.de
Aleksandrow- icz-Trzcińska, M.	Aleksandrowicz-Trzcińska, M. Bogusiewicz, A. Szkop, M. Drozdowski, S.	Effect of chitosan on disease control and growth of Scots pine (Pinus sylvestris) in the forest nursery	marta_aleksandrowicz_trzcinska@ sggw.pl
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Oral Communications

Alonso Chavez, V.	Alonso Chavez, V., van den Bosch, F. , Par- nell, S.	Strategies for monitoring of invasive tree epidemics in tree nurseries for disease detection and tracking
Capretti, P.	Capretti, P., Migliorini D., Botella L., Luchi N.	Northward migration of Gremmeniella abietina from Italian peninsula
Luchi, N.	Luchi, N., Capretti P., Migliorini D., Santini A.	Caliciopsis canker: a damaging disease on pine plantations in Italy
Munck, I.A.	Munck, I.A. Broders, K. Wil- liam Livingston, W. Lombard, K. Luther, T. Ostrof- sky, W.	Extent and severity of Caliciopsis canker in New England, U.S.A: an emerging disease of eastern white pine
Woodward, S.	Fraser, S., Brown, A. Woodward, S.	Aneurinibacillus migulanus Nagano controls Dothistroma Needle Blight of Pine
Jalkanen, R.	Jalkanen, R.	Variation in Susceptibility of Scots and Lodgepole Pine Provenances to Infection by Dothistroma septosporum
Oskay, F.	Oskay, F., Doğmuş Lehtijarvi, H.T. Lehtijarvi, A.	Notes on needle pathogens on larch in northern Finland
Oskay, F.	Tunalı, Z. Oskay, F. Lehtijärvi, A. Aday Kaya, A.G. Doğmuş Lehtijär- vi, H.T.	Impact of boxwood blight pathogens; Cylindrocladium buxicola and Pseu- donectria buxi on Buxus balearica and Buxus sempervirens
Doğmuş-Lehtijarvi, H.T.	Doğmuş-Lehtijar- vi, T., Yeltekin, Ş. Aday Kaya, A.G. Lehtijarvi, A.	In planta detection of needle pathogens in Pinus brutia needles
Doğmuş Lehtijärvi, H.T.	Lehtijärvi, A. Doğmuş Lehtijär- vi, H.T. Oskay, F. Woodward, S.	Disease Severity of Diplodia sapinea on Some Pine Plantations
Aday Kaya, A.G.	Aday Kaya, A.G., Lehtijärvi, A Tunalı, Z. Yeltekin, Ş. Doğmuş-Lehti- järvi, T.	Dothistroma septosporum in Turkey
Aday Kaya, A.G.	Aday Kaya, A.G., Karakaya, A. Doğmuş-Lehtijar- vi, T. Lehtijärvi, A. Oskay, F.	Fungal Endophyte Communities in Needles Differ Between Endemic and Exotic Pine Species in Nearby Plantations in Turkey

S. Fraser	S. Fraser, Brown A. Woodward, S.	Diseases of Coniferous and Deciduous Seedlings in Forest Nurseries in Marmara Region in Turkey
Stanosz, G.R.	Stanosz, G.R., Smith, D.R. Albers, J.	Inter- and intra-specific variation in susceptibility to dothistroma needle blight in Britain. How susceptible are Pinus sylvestris and Pinus contorta?
Michelle Cleary	Michelle Cleary, Diem Nguyen, Diana Marčiu- lynienė, Anna Berlin, Rimvys Vasaitis, Jan Stenlid	Outbreak of needle blight diseases in Pinus radiata: emerging forest diseases in the North of Spain
Samils, B.	Samils, B. Barklund, P. Kait- era, J. Stenlid, J.	The shoot blight and canker pathogen Diplodia scrobiculata and asympto- matic seedlings in natural stands of Pinus banksiana
Dvořák, M.	Dvořák, M. Rotková, G.	Hymenoscyphus fraxineus on Fraxinus mandshurica in Far East Russia: genetic diversity, associated fungal community and evidence of endophytic lifestage
Chastagner, G.A.	Chastagner, G.A. Riley, K. Coats, K. Talgø, V.	Selection and testing Fraxinus excelsior genotypes for resistance against Hymenoscyphus fraxineus in Sweden
Dunnell, K.L.	Dunnell, K.L., LeBoldus, J.M.	Spatial distribution of fungal communities in the stems of healthy and de- clining Populus species commonly used for bioenergy resource in Southern Sweden: Implications for productivity
Doonan, J.	Doonan, J. Den- man, S. Pachebat, J.A. Golyshin, P.N. McDonald, J.E.	Population structure of Cronartium flaccidum and Peridermium pini in relation to the current epidemic
Prieto-Recio, C.	Prieto-Recio, C., Martín-García, J., Bravo, F., Diez, J. J	Spore dispersal of Hymenoscyphus fraxineus in the Czech Republic
Martín-García, J.	Zamora, C., Haque, H., Diez, J.J, Martín- García, J.	Grovesiella and Neonectria canker – Two diseases of Abies in the Pacific Northwest United States
Laflamme, G.	Laflamme, G. Rioux, D.	Using Populus nigra to improve hybrid poplar resistance to Septoria canker in the United States
Brown, N.	Brown, N. Par- nell, S. Denman, S. Filipe, J. Jeger, M. and Bosch, F.	Genomic analysis of bacteria associated with Acute Oak Decline
Adamson, K.	Adamson, K., Drenkhan, R., Pu- idet, B., Kalder, R., Hanso M.	Abiotic factors involved in Pinus pinaster decline in the Iberian Peninsula

Martín-García, J.	Martín-García, J. Woodward, S. Vannini, A. Vettraino, A.M. Alves, A. Hantu- la, J. Bragança, H. Capretti, P. Diez, J.J.	Mycorrhization for improvement conifer seedlings quality in Montenegro
Enebak, S.A.	Enebak, S.A., Starkey, T.E.	Occurrence of Dothistroma septosporum on high altitude pine forests in Montenegro
Bezos, D.	Bezos D., Martínez-Álvarez P., Diez J.J., Fernández M.M.	Inoculation tests of Phytophthora alni and P. plurivora on alder seedlings
Pérez-Sierra, A.	Pérez-Sierra, A., Hendry, S., Gorton, C., Lewis, A., Kal- antarzadeh, M., Sancisi-Frey, S., Brown, A.	Two-year survival of Gremmeniella abietina conidia collected on branches left on the ground after pine harvesting
Hrabětová, M.	Hrabětová, M. Havrdová, L. Černý, K.	Monitoring forest diseases and syndromes: the case of Acute Oak Decline
Elvira-Recuenco, M.	Elvira-Recuenco, M., Iturritxa, E., Raposo R.	Invasive pathogens on pines (Pinus spp.) in Northern Baltics
Muñoz-Adalia, E.J.	Muñoz-Adalia, E.J., Fernández, M.M., Diez J.J.	Gene expression in European Ash in response to inoculation with Hymenos- cyphus fraxineus
Martínez-Álvarez, P.	Martínez-Álva- rez, P., Fernán- dez-González, R.A., Sanz-Ros, A.V., Pando, V., Diez, J. J.	COST Action FP1406: Pine pitch canker - strategies for management of Gibberella circinata in greenhouses and forests (PINESTRENGTH)
Stenström, E.	Stenström, E., Gadjieva, R., Ihrmark, K., Stenlid, J.	Soil fumigation techniques used in the production of forest-tree seedlings in the southern United States
Leticia Botella	Leticia Botella, Miloň Dvořák, Eeva J. Vainio, Libor Jankovsky, Paolo Capretti, Jarkko Hantula, Julio J. Diez, Nicola Luchi	Pityophthorus pubescens associated with Fusarium circinatum in northern Spain
Vendula Čermáková	Vendula Čermáková, Jiří Rozsypálek, Aneta Bačová, Libor Jankovský, Leticia Botella	Fungi associated to necrotic galls of Dryocosmos kuriphilus (Hymenoptera; Cynipidae) in north Spain: preliminary results

Romeralo, C.	Romeralo, C., Witzell, J., Romeralo-Tapia, R., Botella, L., Diez, J.J.	Cedars under threat from Sirococcus spp.
Bennet, P.I.	Bennett, P.I., Stone, J.K.	Evaluation of fungicides for control of ash dieback in forest nurseries
Leon, A.L.	Leon, A.L., Chastagner, G.A.	Phytophthora spp. in nurseries and gardening centres in the Czech Republic
Smith, D.R.	Smith, D. R., Stanosz, G. R.	Gemmamyces piceae – a new important threat for Colorado blue spruce cultivation
Green, S.	Nowell, R.W., Green, S., Laue, B.L., Sharp, P.M.	Impact of seed transmission on the infection and development of pitch canker disease
Prospero, S.	Prospero S., Rigling D.	The use of mycoviruses in forest disease management
Sapp, M.	Sapp, M., Lewis, E., Thwaites, R., Kirk, S., Denman, S., Elphinstone, J.	Evaluation of the antagonism produced by fungal endophytes against Fusar- ium circinatum in the field
Sturrock, R.N.	Sturrock, R.N.	Fungi associated with shoot dieback of birch (Betula pendula Roth.) seed- lings in nursery and after outplanting in forest: preliminary results
Nguyen, D.	Nguyen, D., Cleary, M. R., Enderle, R., Ber- lin, A., Stenlid, J.	First report of Rosellinia spp. on seedling of Pinus sylvestris and Picea abies during cold storage in a forest nursery in Sweden
Desprez-Loustau, M.L.	Desprez-Loustau, M.L., Dezette, D., Fabreguettes, O., Massot, M., Piou, D., Marçais, B.	The European race of Gremmeniella abietina hosts a single species of Gammapartitivirus showing a global distribution and possible recombinant events in its history
Talgø, V.	Talgø, V. Skage, J.O. Steffenrem, A. Johnskås, R.	Interactions among temperature, a novel mycovirus and the conifer patho- gen Gremmeniella abietina
Menkis, A.	Menkis, A. Sten- lid, J. Stenström, E.	Occurrence and implications of putative mycoviral dsRNA molecules in the ash dieback causal agent, Hymenoscyphus fraxineus
Hezil, S.	Hezil, S. Chakali, G.	In vivo and in vitro characterization of the pathogenicity of Hymenoscy- phus fraxineus with the mycelial color as excuse
Njuguna, J.W.	Njuguna, J.W., Barklund, P., Stenlid, J.	Antagonistic activity of fungal endophyte filtrates against Gremmeniella abietina infections on aleppo pine seedlings
Millberg, H.	Millberg, H. Hop- kins, A. Boberg, J. Davydenko, K. Stenlid, J.	Throwing the baby out with the bath water? - Any interactions between foliar pathogens and mycorrhizal fungi in Norway spruce seedlings?
Ritóková, G.	Ritóková, G. Shaw, D.	Reproductive Isolation and Population Structure in the Douglas-fir Swiss Needle Cast (SNC) pathogen, Phaeocryptopus gaeumannii, in the U.S. Pacific Northwest

Stenlid, J.	Stenlid, J. Cleary, M. Brand- ström-Durling, M. Elfstrand, M.	The utility and limitations of molecular diagnostic technologies in forest seedling nurseries
Denman, S.	Denman, S. Plummer, S. Sumner, M. Reed, K. Peace, A. Susan, K.	Preliminary analyses of ITS sequence variation among Marssonina leaf spot and shoot disease pathogens of poplars
Bellahirech, A.	Bellahirech, A. Inácio, M. L. Nóbrega, F. Bon- ifácio, L. Sousa, E. Ben Jamâa, M. L.	Evolutionary adaptations of Pseudomonas syringae pv. aesculi to the Euro- pean horse chestnut
Vasaitis, R.	Menkis, A., Östbrant, I-L. Wågström, K. Vasaitis, R.	Using molecular tools to assess establishment and spread of the biological control agent of chestnut blight
Weiland, J.E.	Weiland, J.E. Beck, B.R. Davis, A. Grunwald, N.J. Santamaria, L. Littke, W.R. Browning, J.E. Edmonds, R.L	Virulence of Hymenoscyphus fraxineus isolates from Lithuanian (post-epi- demic) and Swiss (epidemic) populations