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Global change and tree diseases: New threats and new strategies

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

Key words: Introduced pathogens, climate change, tree health, global trade.

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

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Fusarium circinatum is the causal agent of pitch canker on *Pinus* spp. and *Pseudotsuga menziessi*. The use of pathogen-free seeds is the most important means to prevent infections in nurseries and long-distance spread. In this work, different temperature-time combinations of hot water treatment (HWT) were evaluated to reduce *F. circinatum*

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contamination of *Pinus radiata* seeds without compromising seed quality. Specific objectives of the research were: (i) to evaluate the sensitivity of *F. circinatum* isolates to HWT *in vitro*, (ii) to evaluate *P. radiata* seed germination after HWT, and (iii) to assess the effect of HWT on naturally infected seeds of *P. radiata*. Four *F. circinatum* isolates (two of each mating type) were used to evaluate mycelial growth and conidial germination after treatments at 47, 48, 49, 50, 51, 52, 53 and 54°C for 30 or 45 min. Results showed a significant reduction of mycelial growth and conidial germination associated with an increase of treatment temperature and duration time. Mycelial growth was not observed at temperatures above 50 and 51°C for isolates in *MAT*-2 and *MAT*-1, respectively. Differences were observed on conidial germination among the isolates depending on the mating type: At 49°C no conidial germination was observed for *MAT*-2 isolates; however, *MAT*-1 isolates tolerated temperatures up to 52 to 53°C although percentages of germination were very low. *P. radiata s*eed germination was evaluated after treatments at 50, 51, 52, 53 and 54°C for 30 or 45 min. Percentage of germination in non-treated seeds was 90% and, in treated seeds, percentage of germination decreased significantly with increasing temperature and time. Percentage of germination values ranged from 80% at 50°C for 30 or 45 min to 55 to 40% at 54°C for 30 and 45 min, respectively. Reduction of *F. circinatum* contamination was tested on naturally infected *P. radiata* seeds after treatments at 50, 51, 52, 53 and 54°C for 30 or 45 min. HWT effects were evaluated by assessing the percentage of infected seeds plated onto Komada medium. Percentage of infection of non-treated seeds in Komada was above 70% while only 1% of infected seeds were detected after 50°C-30 min, 50°C-45 min and 52°C-45 min treatments. Results obtained indicate that HWT reduce *F. circinatum* contamination of *P. radiata* seeds.

Key words: *Fusarium circinatum,* hot water treatment, *Pinus radiata* seeds.

Phytophthora diseases of chestnut trees in black sea region of Turkey

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

Key words: Chestnut, *Phytophthora*, Black Sea region, Turkey.

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Semiochemicals for monitoring and control of the pine wood nematode vector *Monochamus galloprovincialis* (Coleoptera: Cerambycidae)

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Monitoring and control of *Monochamus galloprovincialis*, the sole known cerambycid vector of the pine wood nematode *Bursaphelechus xylophilus* in Europe, are among the most promising strategies against this disease. Recent research has unravelled the very complex, chemically mediated, reproductive behaviour in *M. galloprovincialis.* Males and females locate suitable hosts for feeding and oviposition by responding to host and bark beetle semiochemicals. Then, a male emitted pheromone increases the chance for both sexes to meet on the host tree and contact chemical recognition of females is performed before mating. Such findings led to the development of a very efficient attractant lure, consisting in a blend of the aggregation pheromone plus the kairomones, which is already available to researchers and managers. There exist many potentialities of this tool in pine wilt disease integrated management programs along Europe. Determination of dispersal range and mass trapping of the sawyer are among the most relevant. Two dispersal experiments based on the recapture of marked-released adult insects were carried out in natural and reforested pine stands. Recapture in the pheromone-kairomone baited traps was highly successful, accounting for 33.9 and 29.5% of the 174 and 350 released beetles, respectively. Both experiments showed that most insects have low/moderate dispersal, as some 75% of the beetles were recaptured less than 100 or 141 m away, but 6.7% of them were caught at 500 and 6.86% more than 760 m away. One beetle was caught 1.5 km from the release point in the first experiment. Time elapsed between the release and recapture was extended to 91 and 84 days respectively, showing a high life span for *M. galloprovincialis* adults. Results on one mass trapping experiment to test the rate of population extraction and on an operational mass trapping program aimed to vector eradication in the 2008 focus at Sierra de Dios Padre (Extremadura, Spain) are also commented.

Key words: Pine wilt disease, *Monochamus galloprovincialis*, semiochemicals, aggregation pheromone, insect dispersal.

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Effects of leaf spotting caused by mycosphaerella leaf disease and eucalyptus rust on *Eucalyptus globulus* in Uruguay

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

Key words: Mycosphaerella leaf disease, Eucalyptus rust, leaf damage, *Eucalyptus globulus*.

Effects of pruning on pitch canker disease in *Pinus radiata* plantations

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Fusarium circinatum (Niremberg and O´Donell, 1998) is the causal agent of pitch canker disease (PCD) in *Pinus*

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

Key words: Pruning, *Fusarium circinatum*, Cantabria, Monterey Pine, Spain.

Can global warming affect the survival and impact of *P. alni* subsp. *alni*?

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The winter survival of the invasive pathogen *Phytophthora alni* subsp. *alni* in black alder stems was studied after two different winter seasons: cold one in 2008/2009 with an average temperature of –1.96°C and extremely mild one in 2006/2007 with an average temperature of 2.54°C. The difference resembles the expected potential climate change in Central Europe in this century. After the cold winter of 2008/2009, the pathogen survived in only 13.91% samples with an average survival rate of 2.70%. After the mild winter of 2006/2007, the pathogen survived much better and was successfully isolated from 86.09% samples with an average survival rate of 25.52%. Moreover, the total thickness of the covering tissues and exposure to the most heated south western quadrant of stem girth positively affected the pathogen survival. In laboratory experiment with incubation of ten isolates in different temperature and frost duration conditions; the influence of temperature, frost duration and their interaction on *P. a. alni* viability was confirmed ($p < 0.01$). The temperature –0.1 and –2.5°C did not have significant effect on the viability after 4 week incubation. The pathogen viability significantly decreased ($p < 0.01$) when the temperature –5.0°C persisted at least one week. At –10.0°C; no isolate survived till 3 days. The pathogen was very sensitive to hard frost – its median survival time (t_{50}) was only 1 day at –10°C. The survival of *P. a. alni* importantly depended on the temperature. Likely, the pathogen will react to the climate change by having a higher survival rate in aerial tissues and surface roots and a higher disease impact in its existing locations. Likely, the pathogen escapes the influence of hard frost in continental Europe by surviving in roots in non-frozen water and soil.

Key words: *Phytophthora alni*, winter survival, temperature, frost, phytophthora alder disease.

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Assessment of the early effects of climate change on forest health

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

Key words: Global warming, pathogen damage, disease outbreaks.

Fungi in shoots and foliage of *Fraxinus excelsior* and *Fraxinus angustifolia* in Eastern Ukraine

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During the last years, the massive dieback of ash (*Fraxinus* spp.) caused by *Hymenosyphus pseudoalbidus* is observed over large areas of Europe. Until 2010, there was little concern regarding ash health condition in Ukraine, as no dieback symptoms were reported. During the last year, however, some morphological symptoms of ash decline have been observed in the eastern part of the country: Uneven flushing, occasional shoot necroses, discoloration of wood and

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premature leaf-shedding. The main aim of our work was to investigate fungal communities in necrotic and healthy-looking shoots, and in petioles of leaves that were shed during previous vegetation season. The detection of fungi has been accomplished using molecular methods: direct extraction of DNA from plant tissue, its amplification using polymerase chain reaction (PCR) with internal transcribed spacer (ITS) primers, subsequent sequencing, and comparison of the sequences with the sequences of fungi originating from disease-devastated areas. The 176 samples symptomatic and healthy (that is, having necrotic lesions) of shoots and leaves were collected for molecular identification of fungal community and isolation of fungal cultures. In addition, health condition of different ash provenances was visually assessed on seven monitoring plots (24 test trees in every plot) at two locations in eastern Ukraine. The plots represented ash stands from different ages (from 10 to 80) with different crown condition.

Key words: *Hymenosyphus pseudoalbidus*, ash, dieback, ITS primers, crown condition.

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

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Virulence of Turkish *Gremmeniella abietina* isolates was investigated in a field experiment. Five isolates obtained from dead branches of *Pinus nigra* subsp. *pallasiana* and *Pinus sylvestris* in high altitude mountainous areas in the Black Sea Region and the Lakes District were used. The lower branches of 15 to 20 year-old *P. nigra* and *Cedrus libani* in a plantation site at 1050 m a.s.l. in Isparta, were inoculated at 1 to 2 month intervals during September to January. Each isolate was inoculated into one branch per tree and repeated ten times on both tree species at each inoculation date; a total of eighty trees and four hundred-eighty branches were inoculated. The branches were harvested at the end of February, after 166, 112, 64 and 33 days of incubation and lesion length in the inner bark was measured (inoculation wound excluded). The mean lesion length on *P. nigra* and *C libani* were 10.6 ± 0.8 and 3.8 ± 0.2 mm, respectively. In general, differences in the mean lesion length between the isolates were small. Nevertheless, there were significant differences between the isolates on *P. nigra* in November and January inoculations, and on *C. libani* at all four inoculation times. The mean lesion length for all isolates was the highest ($p < 0.05$) in December inoculations for both *P. nigra* (22.0 ± 1.9) and *C. libani* (5.6 ± 0.7). There was no difference between the September and January inoculations on *P. nigra,* despite the almost six-fold difference in incubation period. On *C. libani,* in contrast, the shortest necroses were found in January inoculations ($p < 0.01$). During the December inoculations, the trees were most likely in winter dormancy, which would explain the large lesions.

Key words: *Gremmeniella abietina, Pinus nigra* subsp. *pallasiana, Cedrus libani*.

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Powdery mildew fungi on some deciduous tree species in Turkey

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Powdery mildew is quite a common disease that appears as a white powdery substance occurring on the leaf surface, stem and fruits of many deciduous tree species in European forests. As a result of heavy infection, leaves and affected parts of the tree frequently are distorted and in worst cases infected trees may exhibit symptoms of defoliation or decline. In this study, some deciduous tree species such as *Platanus orientalis, Quercus vulcanica, Quercus robur, Castenea sativa* were inspected for the occurrence of powdery mildew. Additionally, infection rates and distribution were studied from the visible symptoms of the disease observed on *P. orientalis* saplings from seed orchards of Çınarcık, Yalova. *Q. vulcanica* which is an endemic oak species of Turkey shows disease symptoms every year in Kasnak Oak National Park in Isparta Province together with *Q. robur*. *C. sativa* has also powdery mildew infections observed abundantly in one of the recreation areas in Isparta. As a result of macroscopic and microscopic studies, several different fungal species causing powdery mildew on these hosts were found. Mainly characteristics of cleistothecia were used for the identification of the powdery mildew fungi. While *Microsphaera alphitoides* was common fungus on the upper surface of *Q. vulcanica* and *Q. robur*, only *Phyllactinia roboris* which is known as a rare species was detected on the lower surface of the leaves of *Q. vulcanica*. Sweet chestnut and plane trees were infected by *Phyllactinia guttata* and *Microsphaera platani*, respectively. The infection rate and the distribution of *M. platani* on 100 *P. orientalis* saplings were investigated. The saplings were approximately 2.5 cm in diameter and 160 cm in height. In each tree, three topmost lateral shoots and the terminal shoot were checked. All leaves of each shoot were counted and investigated for the presence of the fungus. While all *Platanus* seedlings were found to be infected with *M. platani*, the disease was more common on terminal shoots (85.8%) than on the lateral ones (52.4%).

Key words: Forest disease, *Microsphaera alphitoides*, *Phyllactinia roboris, Phyllactinia guttata, Microsphaera platani*.

Dothistroma septosporum: Incidence of spore production and weather condition

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Dothistroma septosporum (Dorog.) Morelet. and *Dothistroma pini* Barnes are fungal pathogens responsible for a very

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serious needle disease Dothistroma needle blight (DNB), also known as Red band needle blight. DNB was previously considered as a disease supported by global climate change. The influence of locally enhanced precipitations to the spreading of the disease in temperate zone was emphasized. Although a number of papers were published about phenology of DNB, no attempt was made to describe the weather conditions, which are necessary for production of conidia and general pattern of conidia releasing during the year. The aim of this study was to examine the temporal spore dispersal patterns of *D. septosporum*. Spore trapping was done for a period of two seasons from March to December 2009 and 2010 in a 13 year old (2009) plantation of austrian pine (*Pinus nigra*) in the forest district "Soutok", near Lanžhot, South Moravia, Czech Republic. This plantation was strongly infected by *D. septosporum* for a few years. For trapping the Dothistroma spores the automatic volumetric spore trap of usual Hirst, Burkard or Lanzoni VPPS construction was used. Our spore trap (AMET Velké Bílovice, Czech Republic) was placed inside the plantation with the orifice 0.3 m above soil level. Close to the spore trap, a SIGNALIZATOR automatic climatic station (AMET Velké Bílovice, Czech Republic) was installed 2 m above the ground. From the evaluation of sticky tapes from the trap was noticed, that the production of spores occurred solely during days with average day temperature above 10°C and only in the part of season without frost days. Such conditions started in the third decade of April and finished at the beginning of October. During the spore-active season some periods of interruptions were apparent. The longest period without any spore was the first and the second decade of June. It is characterized by average daily temperature above 18°C and average daily relative humidity under 75%. Upon such dry conditions the sporulation ceases within two days. On the other hand, the optimal weather conditions are average daily temperatures 15 to 20°C and average daily relative humidity above 90%. This optimal period was taken from the end of July to the half of September. The highest amount of spores in one cubic meter of air was 3.89. The lowest detectable amount was 0.07 spores/m^3 of air.

Key words: Spore trap, conidia releasing, Dothistroma needle blight, weather.

Phytosanitary conditions of *Quercus cerris* in tuscany evaluated by monitoring, geographic information system (GIS) applications and molecular techniques

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Data from the phytosanitary monitoring program in Tuscany (META) collected during the last four years showed an increasing number of *Quercus cerris* stands affected by *Biscogniauxia mediterranea* (from 12 to 78%) followed by a two-years period of decreasing values. Since the occurrence of this fungus is considered as a good indicator of oak stress in central Italy, a study was performed to verify whether environmental parameters are involved in oak decline. The study was based on the use of geographic information system (GIS) technology and was validated as fungal presence from symptomless oak twig samples. Fungal detection was assessed by using isolation and real-time polymerase chain reaction (PCR) already optimized from this research group in a *B. mediterranea*/*Quercus* pathosystem. During summer

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2009 geographical and monitoring data were obtained from twenty Oak areas in Tuscany. Stands dendrological information and crown conditions evaluated according to international co-operative programme (ICP)-Forest monitoring method were registered; damages and severity index for crown damages were also calculated. Finally, all data were stored and managed by a GIS system by ArcMap 9.3 software. Using climatic data obtained from official local meteorological networks, the mean temperatures and total rainfall during the vegetative seasons were calculated and then data points were interpolated by using Ordinary Kriging technique to obtain prediction climatic maps at local scale. Vector maps of chemical, physical and biological properties of soil according to United Sates Department of Agriculture (USDA) classification, digital elevation model, viability map and *Q. cerris* regional distribution map were lastly stored into GIS system. Results of monitoring showed that the most common damages observed were diebacks and necrosis of branches. Kruskal-Wallis test allowed grading the areas on the base of severity of crown damages level. Results of laboratory analysis confirmed that samples collected from damaged areas hosted a significantly higher amount of *B. mediterranea* DNA in oak asymptomatic tissue compared to areas with lower damages. All geographical and climatic data stored in GIS system were used as predictors. Temperature and soil characteristics showed inverse relationship with crown damages in multiple regression model, while rainfall, elevation, and quantitative of clay and sand were positively linked to crown damages.

Key words: *Biscogniauxia mediterranea*, oak decline, real time PCR, ICP-forest, severity, crown damages.

Use of *Chondrostereum purpureum* in controlling hardwood sprouting

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

Key words: *Chondrostereum purpureum*, biological control, hardwood sprouting, forest regeneration, Heterobasidion root, butt rot.

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Susceptibility assessment of common alder seedlings to *Phytophthora alni* and other *Phytophthora* species

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

Key words: *Alnus glutinosa*, inoculation, susceptibility, *Phytophthora alni*, *in vitro*.

Sequencing and assembly of a fungal genome

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

Key words: Illumina Solexa, fungi, genomic, reads.

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Enhancing systemic resistance of maple against tar spot disease

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

Key words: Induced systemic resistance, systemic acquired resistance, *Acer*, tar spot.

The potential of soil bacteria and their biosurfactants to suppress *Phytophthora* diseases of forest trees

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Diseases caused by the fungi-like organisms, *Phytophthora* spp. (Oomycetes), are a major problem in deciduous forests. Alarmingly, problems with *Phytophthora*-diseases are expected to be further magnified and intensified in the future, since the conditions under the predicted climate change are likely to favor the growth and spread of these pathogens also in the Northern Europe. New control strategies are thus urgently needed. The use of microorganisms as biocontrol agents is an environmentally sound alternative to chemical pesticides and could be an integrated part of sustainable management of *Phytophthora* pathogens of forest trees. A group of microorganisms with high potential in biocontrol are the fluorescent pseudomonads (Pseudomonas spp.). They are indigenous in the environment and may excrete metabolites that are inhibitory to pathogenic microbes. An important group of metabolites produced by fluore-

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scent pseudomonads are biosurfactants. Due to their zoosporicidal activity, these chemicals have attracted increased attention as a potential tool in biocontrol of pathogens belonging to Oomycetes. We initiated studies to evaluate the potential of biosurfactant-producing pseudomonads to protect deciduous forest trees against *Phytophthora* spp. Firstly we used in vitro tests to validate that biosurfactants produced by a fluorescent pseudomonad, *P. koreensis* 2.74, isolated from a horticultural system would effectively lyse the zoospores of oak pathogenic *P. quericina.* Secondly, we conducted a greenhouse experiment with young oak seedlings to study whether the mortality of oak seedlings due to *P. quercina* infections could be reduced by treating the plants with biosurfactant-producing pseudomonads. Thirdly, we investigated whether the treatment with bacteria could also induce alterations in the potentially defensive phenolic metabolites in oaks. The implications of the results for control strategies in nurseries and natural habitats were discussed.

Key words: Biocontrol, sustainable management, *Pseudomonas spp.*, phenolic metabolites.

New alternate hosts for *Cronartium* spp. in Finland

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

Key words: Alternate hosts, *Cronartium flaccidum, Cronartium ribicola, Pinus sylvestris,* scots pine blister rust, white-pine blister rust.

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

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

Key words: Brown spot needle blight, Austria, spread of disease, host range, *Mycosphaerella dearnessii, Lecanosticta acicola, Dothistroma septosporum*.

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Phytophthora-infection in a sweet chestnut (*Castanea sativa*) Orchard in Transdanubia, Hungary

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In the first decades of the 20th Century, a big amount of sweet chestnut (*Castanea sativa*) which stands in the South-Europe died. The cause was the ink disease. Since then, the ink disease is one of the most serious diseases of the sweet chestnut in the whole Europe. There are several *Phytophthora* species in contact with the appearance of the ink disease. The most frequent species are *Phytophthora cambivora* and *Phytophthora cinnamomi*. The appearance of ink disease is sparse in Hungary. In September 2010, we found dying trees in a sweet chestnut orchard of 14 year old in South-Transdanubia, Hungary. The symptoms were specific: small, yellowish leaves, sparse, drying foliage, necrotic bark lesions at the stem basis and main roots. The fine roots were rot. Some saplings also died in the same orchard. We isolated *Phytophthora* species on selective agar media from soil samples taken from the rhizosphaere of the dying trees and saplings by baiting with *Rhododendron* leaves. The species identification was carried out by morphologic examination and the internal transcribed spacer (ITS1) and ITS2 sequences of the ribosomal DNA. We identified *P. cambivora* from the soil of dying trees and of saplings, too. Now, the appearance of ink disease in the orchard is confined to a small clump. It is an urgent task to find the adequate methods to confine the disease.

Key words: Ink disease, internal transcribed spacer, ribosomal DNA, *Phytophthora cambivora*.

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White pine needle diseases in Eastern Canada

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In 2009, yellowing of white pine (*Pinus strobus*) needles was reported from several regions in three Canadian provinces: New Brunswick, Québec and Ontario. A similar problem was seen also in eastern United States. Several causal agents

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

Key words: *Pinus strobus, Lophophacidium dooksii, Mycosphaerella dearnessii*, needles diseases.

Seasonal variation in the infection level of *Cedrus libani* needles by *Ploioderma cedri*

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

Key words: Cedar forest, Turkey, *Ploioderma cedri*, disease.

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Neonectria sp., a new pathogen causing cankers on Norway spruce?

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In 2001, dieback and cankers with resin flow were found in a Norway spruce stand in Saarijärvi. A trial with one progeny from Poland was established by breeders in 1985. In a second evaluation in 2001, breeders noticed that several trees had their tops dying and in addition they had strange, black wounds on their trunks. The trial was thinned and dying trees were removed in 2003. In 2008, the trial was assessed again and 13% of the trees in Finnish families had wounds while 37% of the trees were damaged in the Polish provenance. In 2005, similarly damaged trees were found in other progeny trials and plantations. In all trials, trees representing southern origin had more damages than Finnish spruces. More recently, 5 to 30 year-old stands suffering from the same kind of cankers and necrotic lesions were found in private forests in different parts of Finland. In most cases, the origin of Norway spruce seedlings used for planting has not been possible to trace. The same disease has been reported from Norway on white fir, Siberian fir, subalpine fir and also on Norway spruce, especially in south eastern Norway. Fungal isolations done in Finland and Norway resulted in several fungi, but the only common fungal genus was *Neonectria*. The sequencing of the internal transcribed spacer (ITS) of ribosomal DNA showed that most isolates from Finland were identical to *Neonectria fuckeliana,* while the Norweigian isolates were most similar to *N. ditissima* (*N. galligena)*. In inoculation tests both species were pathogenic.

Key words: Progeny, necrotic lesions, resin flow, Finland, Norway.

Transmission of *Diplodia pinea* via the new invasive insect *Leptoglossus occidentalis*

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

Key words: Global warming, invasive insect species, real time PCR, fungal pathogen.

Red band needle blight – Molecular screening of the Czech Republic

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

Key words: *Dothistroma septosporum*, molecular markers, polymerase chain reaction.

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

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

Key words: Endophytic fungi; environmental variables; soil properties; crown condition; dendrometric variables.

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

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The European black poplar (*Populus nigra*) is considered as one of the most seriously endangered indigenous tree species in Europe. The question remains whether a possible extinction of *P. nigra* through replacement by *Populus x euramericana* or a possible drastic decrease in genetic variation through intensive introgression would imply the extinction of those organisms that are obligatory associates of *P. nigra*. To achieve this, we compared the endophytic mycota in twigs of native poplar (*P. nigra*) and hybrid poplar plantations (*P. x euramericana* clone I-214). Twig endophytes were isolated from native and hybrid poplar stands in Palencia (N. Spain), and identified according to

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sequences of the internal transcribed spacer (ITS) region of their rDNA. The results reveal that the endophyte community on poplar forests may be affected by an extinction of *P. nigra* or a drastic decrease in genetic variation, because it differed considerably between native and hybrid poplars of this study.

Key words: Native, hybrid, poplar, endophyte, twig.

Changes of the shrub structure in a Turkey-oak forest after a tree decline in North-Hungary

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Similar to other European countries there was an oak decline at the end of the 70's in Hungary; 68% of the *Quercus petraea* specimens of the forest's trees died at the site. This decline resulted in an opening of the canopy; the canopy cover decreased from 80% (1972) to 36% (2007). The aim of our study was to analyse the structural changes in the forest interior after oak decline. We focused on those specimens which were higher than 8 m (so-called secondary trees). Height and diameter of these specimens were registered; their location and cover percentage were mapped from 1972. We focused on the following questions: (1) What are the most important structural changes in the forest interior after the oak decline? (2) Which woody species are the most successful shade-tolerant species and what are the possible ecological reasons of this process? In 2007, there were 130 specimens of *Acer campestre*, 22 specimens of *Cornus mas* and 4 specimens of *Acer tataricum* between 8 - 13 m height and created a secondary tree layer below the primary tree layer of oaks. The mean cover of these species increased remarkably after the tree decline. The canopy cover was 32% of high shrub layer cover in this newly formed layer. Our study reveals that the forest responded to the oak decline by structural changes in the shrub layer and three woody species of secondary tree layer compensated the remarkably loss of tree canopy cover.

Key words: Oak decline, *Acer campestre*, *Quercus petraea*, density, canopy cover, secondary tree layer.

Does long distance gene flow occur between subpopulations of *Lophodermium piceae*, the most common needle endophyte of Norway spruce?

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

Key words: Genetic differentiation, SCAR, DNA markers, fungi, ascomycete.

Monitoring damage from foliage, shoot and stem diseases in New England and New York.

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

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unusually wet spring weather. Results from forest health monitoring efforts are made publicly available via web-based products such as the Forest Pest Portal (http://www.foresthealth.info/) and Forest Health Highlights (http://fhm.fs.fed.us/fhh/fhmusamap.shtml).

Key words: Forest health, detection, invasive, exotic, alien pests

Impact of Beech bark disease on the sustainability of American Beech in New York

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

Key words: Nectria, forest structure, landscape-scale analyses, invasive, exotic, alien pests.

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Epidemic and pathogenicity of *Chalara fraxinea* causing ash dieback in Hungary

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

Key words: *Chalara fraxinea*, *Fraxinus*, susceptibility, inoculation.

Red pine logging debris as a potential source of inoculum of *Diplodia* shoot blight pathogens

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Following the observation of a high incidence of *Diplodia* shoot blight on recently planted red pine (*Pinus resinosa*) seedlings growing where mature red pine stands previously had been clearcut, the potential of logging debris as a source of inoculum of *Diplodia* pathogens was investigated. Cones, bark, needles, stems from shoots bearing needles, and stems from shoots not bearing needles (both suspended above the soil and in soil contact) were collected from debris left at sites that were previously clearcut. Conidia were extracted in water, quantified, tested for germinability, and *Diplodia* species were identified from samples using a polymerase chain reaction (PCR) assay. Conidia of *Diplodia*

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species were detected from all study sites. Repeated sampling of the same sites at 6, 12, and 18 months postharvest revealed an initial increase in the numbers of conidia. Although fewer conidia were obtained from debris collected from additional sites at greater intervals since harvest (and fewer of these germinated), all substrates yielded many germinable conidia even 5 years postharvest. The type of host substrate from which conidia were extracted had an effect on the number of conidia quantified and the percentage of conidia that germinated. Also, more conidia were obtained and a greater percentage germinated from debris that was suspended above soil at the time of collection than from debris in soil contact. Because red pine seedlings are commonly planted in close proximity to logging debris on clearcut sites and germinable conidia were abundant, debris could be a potential persistent source of inoculum for *Diplodia* shoot blight pathogens to planted seedlings. The results of this study should prompt further consideration by land managers of the potential forest health risks, in addition to benefits, that may be associated with logging debris.

Key words: Conidia, debris, *Diplodia, inoculum*, pine, *Pinus resinosa*, *Sphaeropsis*.

Cedrus libani - A new host for *Herpotrichia juniperi*

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

Key words: *Cedrus libani*, brown felt blight, *Herpotrichia juniperi*, Turkey, high elevation forests.

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Evaluation of genetic resistance to *Fusarium circinatum* in *Pinus* species

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

Key words: Pine, Spain, inoculation, susceptibility.

Effects of temperature, pH and osmotic potential on *in vitro* mycelial growth of *Gremmeniella abietina* isolates infected by mitoviruses

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

Key words: Mitoviruses, Scleroderris canker, *in vitro*, *Gremmeniella abietina*.

Investigations on *Phytophthora plurivora and Phytophthora pini* in Finland

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

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present in Finnish nurseries has been found in natural ecosystems. As *P. plurivora* has been very recently encountered on many tree species in semi-natural ecosystems in other Scandinavian countries, further screening for this tree pathogen in addition to other *Phytophthora* spp. will be conducted in Southern Finland in 2011.

Key words: Oomycetes, nursery, plant trade, oogonia.

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Research on Oak decline disease in Spain

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

Key words: Holm oak, cork oak, *Phytophthora cinnamomi*, soil moisture.

Interaction between *Gremmeniella abietina* and several fungal endophytes

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

Key words: *Gremmeniella abietina*, endophytes, antagonism, *in vivo*, pathogenicity.

Analysis of factors influencing canopy loss in pine stands infected by *Gremmeniella abietina* in Northern Spain

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In this study, the influence of environmental and silvicultural factors on canopy condition was evaluated in *Pinus halepensis* stands from Northern Spain where *Gremmeniella abietina* infections have been reported. Canopy condition was evaluated estimating defoliation and leaf area index (LAI). Hemispherical photography analysis was used as an

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indirect method to estimate LAI, and International Co-operative Programme for the Intensive Monitoring of Forest Ecosystems (ICP-Forest) methodology was used for defoliation estimation. Several environmental and stand parameters were measured in order to test their influence on canopy condition. Multivariate and multiple regression analyses were carried out to identify the main factors influencing defoliation and LAI. *G. abietina* has been found to produce severe damages on *P. halepensis* in this area, and its activity is influenced by several factors such as canopy depth, age, basal area and elevation. On the other hand, LAI is determined by mean diameter, tree density and canopy openness. The relationship between defoliation and LAI was very weak and factors affecting both parameters were different. The way in which *G. abietina* produces dieback and defoliation, being the latter heterogeneously distributed through the canopy (affecting mainly the upper part of the canopy) decrease the correlation between them. The contrary was found for other defoliating agents, such as after some insects outbreaks, in which this relationship was stronger and clearly proved. This suggests that symptoms produced by pathogens causing dieback will be not detected just estimating LAI, being necessary a visual evaluation for this purpose.

Key words: *Gremmeniella abietina*, defoliation, dieback, leaf area index, LAI, hemispherical photography, *Pinus halepensis*.

Effect of temperature on survival of *Fusarium circinatum*

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

Key words: Needles, wood, inoculation, *Pinus radiata*.

Distribution of *Mycosphaerella* leaf disease on Eucalyptus in Portugal

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

Key words: *Mycosphaerella*, *Teratosphaeria*, leaf disease, MLD, *Eucalyptus*.

Pathogenicity trials with *Gremmeniella* fungi collected on conifers in Canada

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

Key words: Scleroderris canker, *Gremmeniella* spp., *Abies balsamea, Picea* spp.

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Storage conditions influence cultural detection of the shoot blight pathogen *Diplodia pinea* on or in asymptomatic red pine nursery seedlings

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

Key words: *Diplodia pinea*, pine, *Pinus resinosa*, seedling, *Sphaeropsis*, storage.

Cultural detection of *Diplodia* shoot blight pathogens from red pine and Jack pine seeds

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

Key words: *Diplodia pinea, Pinus banksiana, Pinus resinosa*, pine, seed, *Sphaeropsis*.

Expansion in the known geographic distribution and host range of the shoot blight pathogen *Sirococcus tsugae*

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Eastern hemlock (*Tsuga canadensis*) is an ecologically and economically important conifer from the north-central United States to the east coast of North America to the southern Appalachian Mountains. In early spring 2010, blighted shoot tips of eastern hemlock were observed at widely separated locations in the Chattahoochee National Forest in north Georgia. Damage did not appear to be directly related to hemlock woolly adelgid (*Adelges tsugae*) activity, which was sporadic or absent in some areas where symptoms were observed. A preliminary survey in March 2010 revealed that incidence of blighted shoots on individual trees varied, but was as high as 70%. Stems of shoots produced the previous

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year were frequently necrotic, had lost needles, and bore pycnidia with hyaline, two-celled conidia consistent with those of *Sirococcus tsugae*. Later in the spring and summer, shoots of the current year's growth became blighted, with sporulation of *S. tsugae* also on dead and dying needles. While *S. tsugae* previously has been reported on *T. heterophylla*, *T. mertensiana*, *Cedrus atlantica*, and *C. deodara* in western North America, it has only recently been reported on eastern hemlock, and its ability to induce shoot blight had not been proven. Pure cultures were obtained on streptomycin-amended potato dextrose agar (PDA) and their identity was confirmed by species-specific polymerase chain reaction (PCR) primers. Nuclear rDNA internal transcribed spacer sequence also was obtained and was identical to sequences for *S. tsugae* previously deposited in GenBank. Two isolates were used to inoculate potted 2-year-old eastern hemlock seedlings in a growth chamber at 20°C with a 16-h photoperiod. Conidia were collected by flooding 1-month-old colonies on PDA with sterile water. Expanding shoots on one branch of each seedling were wounded using scissors to cut the tips off needles and stems, while another branch remained unwounded. Ten seedlings per isolate were inoculated by spraying to runoff with a conidial suspension sterile water, and five similarly treated control seedlings were sprayed with sterile water. Seedlings were covered with plastic bags to maintain high humidity for 4 days. Symptoms were evaluated and reisolation was attempted on streptomycin-amended PDA 2 months after inoculation. Symptoms of seedlings inoculated with either isolate included chlorotic and necrotic needle spots, browning of cut edges of needles, browning and death of needle tips and entire needles, death of stem tips with retention of dead needles, and needle loss. Symptoms of control seedlings were limited to slight browning of cut edges of needles. The fungus was reisolated from wounded shoots of 17 of 20 inoculated seedlings and nonwounded shoots of 5 of 20 inoculated seedlings and was not cultured from control seedlings. To our knowledge, this is the first report of *S. tsugae* in Georgia and also the first demonstration of its ability to produce symptoms that have been attributed to it on any tree species.

Key words: Hemlock, shoot blight, *Sirococcus tsugae*, *Tsuga canadensis*.

Gremmeniella epidemic in Sweden in 1999 and 2001 - Recovering of the forest

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During 1999 and 2001, the most severe *Gremmeniella abietina* epidemics ever appeared in Sweden. More than 500,000 ha forest were severely attacked and the forest industry lost milliards of Euros. In order to follow the development of the forest after one or two *Gremmeniella* infections, we studied seven experimental sites established in the most affected areas in middle of Sweden. In total, we followed the defoliation and growth of 360 trees exposed to two epidemics and of 250 trees exposed to one epidemic. When the experiment started in 2000 and 2001, trees were chosen according to different defoliation: healthy (<20% defoliation), medium (60-70%) or severely defoliated (80-90%). The number of epidemics affected the survival of trees in the medium defoliation class, but not in the severely and healthy classes. When subjected to two epidemics, survival after 10 years of medium defoliated trees was almost similar to that of severely defoliated trees (40% vs. 25%, respectively). When only attacked once, both medium and severely defoliated trees showed a higher survival (65% vs. 35%). After one epidemic, surviving trees presented a lower defoliation (25%)

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than when subjected to two epidemics (50%) that is, less defoliated trees survived. We also observed different patterns amongst surviving trees subjected to one or two epidemics. Severely defoliated trees subjected to one epidemic recovered the growth at a similar rate than trees with medium defoliation. Severely infected trees subjected to two epidemics recovered the growth at a lower rate than trees with medium defoliation. Recurrent epidemics severely diminish the capacity of survival and recovery from *G. abietina* attacks. Knowledge on preceding attacks may be used to optimize tree removal after the epidemics. Trees shall be removed based upon different defoliation thresholds depending on the previous history of the stand.

Key words: *Gremmeniella abietina*, defoliation, growth, survival.

Climate change and forest diseases: Using today's knowledge to address future challenges

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

Key words: Tree diseases, forest pathogens, forest health, urban forests, plant disease management.

Dothistroma septosporum and *Lecanosticta acicola* in Czech Republic: Current situation and inoculation tests

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Severe spreading of Needle blights has been noticed within the past 15 years. Ascomycetes *Dothistroma septosporum* (Dorog.) Morelet (teleomorph: *Mycosphaerella pini* Rostr.) and *Dothistroma pini* Hulbary (teleomorph: unknown) causing Dothistroma Needle Blight (Red Band Needle Blight) and *Lecanosticta acicola* (Thüm.) Syd. (teleomorph: *Mycosphaerella dearnessii* M. E. Barr), causing Brown Spot Needle Blight, are considered as the causal agents. The occurrence of these pathogens from new sites as well as from new host tree species has been reported. Scandinavia can be an example of such crucial spreading of the disease. The Red Band Needle Blight has been occurring in northern Europe since the year 2008 in a big scale mainly on *Pinus sylvestris*. Woods et al. (2005) consider Dothistroma needle blight (DNB) as a disease supported by global climate change. He emphasizes the influence of locally enhanced precipitations to the spreading of the disease in temperate zone. The increasing amount of new infested sites has been noticed also in Czech Republic. The fast disease spreading plays the key role in this current situation. The observation of the disease development has been carried out in 132 sites with host tree species so far. DNB has been confirmed on 79 sites and from 23 of those pure cultures have been successfully cultivated. The occurrence of Brown Spot Needle Blight is not too alarming in the CZ as DNB from the point of the amount of infested sites. Nevertheless Brown Spot Needle Blight in Southern Bohemia occurs in a big scale hence its spreading to new areas is not out of the question. The current occurrence of Needle Blights in a huge scope and its relatively easy and fast spreading has became the main reason of the inoculation spraying test. The purpose of the inoculation test is to verify the symptomology of Needle Blights. The inoculation test will be done simultaneously with the Real-Time polymerase chain reaction (PCR), whereby only a little DNA concentration of the organism in a sample can be detected. By this it is possible to detect an occurrence of a disease in its early stage, which is a problem for diagnostics. The identification of the pathogen in the early stage could help the use of protective spray for prevention of the occurrence and spreading of the disease. Another purpose of the inoculation test is the question of susceptibility of various conifer species on the *Dothistroma* and *Lecanosticta* infection. Inoculation tests have been carried out since spring 2010 and a quarantine greenhouse in Praha-Ruzyne and former Hacker's nursery have been chosen for the purpose of this experiment. Pure cultures of *D. septosporum* and *L. acicola* were used as a basic inoculation suspension, whereas their conidias were washed by double distilled water. Inoculation suspension was applied on seedlings with atomizer. Selected seedlings were consequently covered with unwoven fabric for encouraging favourable microclimatic conditions. Following seedling species have been placed in the greenhouse or/and planted *in natura* in former nursery: *Pinus nigra, Pinus mugo, Pinus sylvestris, Pinus uncinata, Picea pungens, Picea abies, Picea sitchensis and Pseudotsuga menziesii*. With respect to actual epidemic situation in many countries, it is necessary to discuss the role of climatic factors in Europe and trade with plant material as the main risk factor for the spreading of both diseases.

Key words: *Dothistroma septosporum* and *Lecanosticta acicola*, Czech Republic, Dothistroma needle blight (DNB).

Occurrence of *Dothistroma septosporum* in different types of forests in Finland

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Red band needlecast has been found in Finland during the last years. It has been distributed almost all over Finland, but seems to be most common in the southern and central part of the country. The aim of this study was to find out the frequency of red band needle blight distribution in different type of pine stands. Normally the pine stands in dry forest

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sites are healthy without any needlecast. Although the reasons for disease distribution are unclear, the increased length and increased humidity of growing seasons may favour the dispersal and infection of spores. Earlier epidemics caused by *Lophodermium seditiosum* could outbreak especially in southern Finland, but during the last years red band needle blight has also caused needlecast in pine stands, too. The amount of red band needlecast varies a lot between sites, depending on the density of trees vegetation and the type of soil etc.

Key words: *Lophodermium seditiosum*, Finland, needlecast.

Control of chestnut canker with hypovirulent strains of *Cryphonectria parasitica* in Castilla y León (Spain)

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

Key words: Hypovirulence, *Cryphonectria parasticia*, chestnut canker, Castilla y León.