

# **LIFE+ ELMIAS Ash and Elm, and IUFRO WP 7.02.01 Root and Stem Rots Conference (LIFE-IUFRO)**

26 August - 1 September 2018, Uppsala and Visby, Sweden

## **PROGRAM & BOOK OF ABSTRACTS**

*Edited by Rimvys Vasaitis*



**LIFE+ ELMIAS Ash and Elm, and IUFRO WP 7.02.01 Root and Stem Rots  
Conference (LIFE-IUFRO)**

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PROGRAM & BOOK OF ABSTRACTS

*Financial support for organizing the Conference activities on Gotland Island (venue, excursions, catering) was provided by the EU Project LIFE+ ELMIAS - Saving wooded Natura 2000 habitats from invasive alien fungi species on the Island of Gotland, Sweden (LIFE12 NAT/SE/001139). The support is gratefully acknowledged.*



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# TALK PRESENTATIONS IN UPPSALA

## August 26<sup>th</sup>

~~16:00 – ... reception at Uttuna Campus; CANCELLED~~

## August 27<sup>th</sup>

08:00 – 08:50; arrival, registration, hanging out posters;

08:50 – 09:00; welcome address by RV & JS;

### SESSION 1 Chair: PAOLO GONTHIER

09:00 – 09:20; **presentation 1; Species, distribution and spore density of *Heterobasidion* in Canada**

J.A. BÉRUBÉ, A. POTVIN, D. STEWART, P.N. GAGNÉ, J.P. PONCHART, J. PHELAN, A. VARGA, D. JAMES, E.D. TREMBLAY, M.O. DUCEPPE, T. KIMOTO, G.J. BILODEAU

09:20 – 09:40; **presentation 2; Exploration of genetic and silvicultural approaches to protect Norway spruce from root rot caused by *Heterobasidion annosum sensu lato***

E. TERHONEN, G.J. LANGER, J. BUßKAMP, K. BLUMENSTEIN

09:40 – 10:00; **presentation 3; Precision forestry for improved resource utilization and reduced wood decay in Norwegian forests**

A.M. HIETALA, H. SOLHEIM, K.H. HANSEN, B. TALBOT, R. ASTRUP

10:00 – 10:20; **presentation 4; Quo vadis, *Heterobasidion*?**

P. SEDLÁK

### 10:20 – 10:50: COFFEE / TEA BREAK INCL. POSTER PRESENTATIONS

### SESSION 2 Chair: JAN STENLID

10:50 – 11:10; **presentation 5; An inoculation experiment suggests that presence of the native forest pathogen *H. annosum* in European forests may facilitate the invasion by the exotic *H. irregulare*.**

P. GONTHIER, L. GIORDANO, G. LIONE, M. GARBELOTTO

11:10 – 11:30; **presentation 6**; **Assessing the association between fitness of *Heterobasidion irregulare* × *H. annosum* hybrids and the mitochondrial genome**

L. GIORDANO, F. SILLO, M. GARBELOTTO, P. GONTHIER

11:30 – 11:50; **presentation 7**; **Genomic admixing between *Heterobasidion irregulare* and *H. annosum* varies depending on the direction of interspecific introgression and may confer adaptive advantages to both species**

F. SILLO, M. GARBELOTTO, P. GONTHIER

11:50 – 12:10; **presentation 8**; **A new North American *Heterobasidion* genome and the new provisional hybrid species *Heterobasidion irregularicis* from Alpine larch**

M. GARBELOTTO, P. GONTHIER, B. LOCKMAN, F. SILLO

**12:10 – 13:50; LUNCH BREAK INCL. POSTER PRESENTATIONS**

**SESSION 3. Chair: HELI VIIRI**

13:50 – 14:10; **presentation 9**; ***Heterobasidion annosum* root rot in noble fir combined with bark beetle attack**

M.J. JUSTESEN, I.M. THOMSEN

14:10 – 14:30; **presentation 10**; **Perspective of growing *P. contorta* in Latvia: focus on *Heterobasidion* root rot**

A. ZALUMA, I. MUIŽNIEKS, N. BURŅEVIČA, A. JANSONS, L. SISENIS, T. GAITNIEKS, J. STENLID, R. VASAITIS

14:30 – 14:50; **presentation 11**; **Fungal community structure in asymptomatic and symptomatic Norway spruce trees naturally infected by the conifer pathogens *Heterobasidion* spp.**

A. KOVALCHUK, M. MUKRIMIN, Z. ZENG, T. RAFFAELLO, M. LIU, R. KASANEN, H. SUN, F.O. ASIEGBU

14:50 – 15:10; **presentation 12**; **Ecology and distribution of *Heterobasidion* species in Serbia**

N. KEČA, L. KEČA

**15:10 – 15:40: COFFEE / TEA BREAK INCL. POSTER PRESENTATIONS**

#### SESSION 4. Chair: TIIA DRENKHAN

15:40 – 16:00; **presentation 13**; Potential “new” protective agents for biocontrol of *Heterobasidion* spp. on Norway spruce

J. RÖNNBERG, Z. MAGAZNIECE

16:00 – 16:20; **business meeting of IUFRO WP070201**

16:20 – 16:40; **presentation 14**; Development of *Heterobasidion* genets in even-aged Norway spruce stands on peat soils

L. BRŪNA, A. ZAŁUMA, T. PIRI, D. KĻAVIŅA, T. GAITNIEKS

16:40 – 17:00; **presentation 15**; *Armillaria gallica* – distribution and DNA sequence variability

M. TOMŠOVSKÝ, V. ANTONÍN

17:00 – 17:20; **presentation 16**; Spatial distribution of wood decay fungi in Southern Moravian forest types

L.B. DÁLYA, P. SEDLÁK

17:20 – end

### August 28<sup>th</sup>

#### SESSION 5. Chair: ARI HIETALA

09:00 – 09:20; **presentation 17**; A small secreted protein from necrotrophic pathogen *Heterobasidion parviporum* (HpSSP) induces cell death in *Nicotiana benthamiana* and elicits defense responses in Norway spruce

Z. WEN, T. RAFFAELLO, Z. ZENG, F.O. ASIEGBU

09:20 – 09:40; **presentation 18**; Physiological and molecular features of necrosis response in *Heterobasidion*-conifer pathosystem

M. LIU, A. KOVALCHUK, E. JABER, Z. ZENG, F.O. ASIEGBU

09:40 – 10:00; **presentation 19**; Bacterial biota and correlation with host terpenoid profiles of asymptomatic and symptomatic Norway spruce infected by *Heterobasidion* spp.

F. REN, A. KOVALCHUK, M. MUKRIMIN, M. LIU, Z. ZENG, R.P. GHIMIRE, M. KIVIMÄENPÄÄ, J.K. HOLOPAINEN, H. SUN, F.O. ASIEGBU

10:00 – 10:20; **presentation 20**; **Metabolomics and RNA-seq provides novel insights into interactions between Norway spruce and necrotrophic pathogen *Heterobasidion annosum s.l.***

A. KOVALCHUK, Z. ZENG, R.P. GHIMIRE, M. KIVIMÄENPÄÄ, T. RAFFAELLO, M. LIU, M. MUKRIMIN, R. KASANEN1, H. SUN, R. JULKUNEN-TIITTO, J.K. HOLOPAINEN, F.O. ASIEGBU

## 10:20 – 10:50: COFFEE / TEA BREAK INCL. POSTER PRESENTATIONS

### SESSION 6. Chair: ASTRA ZALUMA

~~10:50 – 11:10; **presentation 21**; **Root and stem rots of forest trees in Central Siberia**  
**I. PAVLOV**;~~

~~CANCELLED, BUT THE ABSTRACT RETAINED UNDER THE SAME NUMBER~~

10:50 – 11:10; **presentation 22**; **Resistance breeding against *Heterobasidion spp.* in Norway spruce**

M. ELFSTRAND, Å. OLSON, K. LUNDÉN, H. CAPADOR-BARRETO, R. GARCÍA-GIL, B. KARLSSON, H.X. WU, J. STENLID

11:10 – 11:30; **presentation 23**; **Transgenerational defence priming in *Pinus pinaster***

J. OLIVA, M. ELFSTRAND, K. KRAUSE, S. GIMÉNEZ, M. DE LA FUENTE, L. SAMPEDRO, R. ZAS

11:30 – 11:50; **presentation 24**; **Genome-wide DNA methylation analysis provides insights on regulation of necro-/saprotroph lifestyles and developmental stages in *Heterobasidion parviporum*.**

Z. ZENG, T. RAFFAELLO, M. LIU, Z. WEN, F.O. ASIEGBU

11:50 – 12:10; **presentation 25**; **Resistance of Scots pine trees to *Heterobasidion annosum* infection: gene expression profiling and terpenoid analysis**

M. MUKRIMIN, A. KOVALCHUK, H. SUN, R.P. GHIMIRE, M. KIVIMÄENPÄÄ, J. HOLOPAINEN, F.O. ASIEGBU

## 12:10 – 13:50: LUNCH BREAK INCL. POSTER PRESENTATIONS

### SESSION 7. Chair: EEVA TERHONEN

13:50 – 14:10; **presentation 26**; **Disease control of *Phytophthora plurivora* and other *Phytophthora* species infecting European beech and rhododendron in the United States**

J.E. WEILAND, C.F. SCAGEL, N.J. GRUNWALD

14:10 – 14:30; **presentation 27**; Screening *Quercus suber* and *Q. ilex* for resistance to drought and *Phytophthora cinnamomi*

B. CUENCA, L. LUQUERO, L. OCANA, A. SOLLA

14:30 – 14:50; **presentation 28**; Offspring of ink-diseased chestnut trees in response to water stress and *Phytophthora cinnamomi*

A. CAMISON, M.A. MARTIN, J. OLIVA, M. ELFSTRAND, A. SOLLA

14:50 – 15:10; **presentation 29**; Beech bark disease in Slovakia: history and current situation

O. MIŠÍKOVÁ, V. RAČKO, I. MIHÁL, J. ĎURKOVIČ

### 15:10 – 15:40: COFFEE / TEA BREAK INCL. POSTER PRESENTATIONS

#### SESSION 8. Chair: KATERYNA DAVYDENKO

15:40 – 16:00; **presentation 30**; Emerald ash borer *Agrilus planipennis* Fairmaire, 1888 (Coleoptera: Buprestidae) within its native range in East Asia

D.L. MUSOLIN, A.V. SELIKHOVKIN

16:00 – 16:20; **presentation 31**; *Tomostethus nigritus* sawfly damage on ash in Finland. H. VIIRI, A. POUTTU, I. LUMME, I. MANNERKOSKI

16:20 – 16:40; **presentation 32**; *Scolytus multistriatus* on Gotland Island: phenology and infectiousness with Dutch Elm Disease

A. MENKIS, K. DAVYDENKO, K. WÅGSTRÖM, I.L. ÖSTBRANT, J. STENLID, R. VASAITIS

16:40 – end

### Uppsala, August 29: excursion in mainland Sweden, and boarding the ferry Nynäshamn – Visby

The field trip starts on 29 August, at **08:30**. We will meet up at **08:10**, at the main entrance to Uppsala Biocenter, located about 100m east from the bus stop (on a way to Conference venue).

## TALK PRESENTATIONS IN VISBY

### August 30<sup>th</sup>

08:00 – 08:50; registration of newly attending participants, hanging out posters at the Wisby Strand Conference Center

08:50 – 09:00; introduction by RV & Karin Wågström

#### SESSION 9. Chair: IRYNA MATSIAKH

09:00 – 09:20; **presentation 33**; **Veteranisation – using tools instead of time**  
V. BENGTSSON

09:20 – 09:40; **presentation 34**; **Predicting ash dieback severity and environmental suitability for the disease in forest stands in the Czech Republic**  
E. CHUMANOVÁ, D. ROMPORTL, L. HAVRDOVÁ, D. ZAHRADNÍK, V. PEŠKOVÁ, K. ČERNÝ

09:40 – 10:00; **presentation 35**; **Factors associated with the severity of ash dieback in south-western Germany**  
R. ENDERLE , G. KÄNDLER

10:00 – 10:20; **presentation 36**; **Pragmatic decision-making for NATURA 2000 ashwoods in the face of ash dieback and other threats**  
D. STONE

#### 10:20 – 10:50: COFFEE / TEA BREAK INCL. POSTER PRESENTATIONS

#### SESSION 10. Chair: LOUIS BERNIER

10:50 – 11:10; **presentation 37**; **Evaluation of the impact of *Hymenoscyphus fraxineus* in ash stands in Serbia**  
N. KEČA, L. KEČA

11:10 – 11:30; **presentation 38**; **Consideration of traits that facilitate invasibility of *Hymenoscyphus fraxineus* in Europe**  
A. M. HIETALA, I. BORJA, N. E. NAGY, H. SOLHEIM, V. TIMMERMAN

11:30 – 11:50; **presentation 39**; **Variation in susceptibility to ash dieback caused by *Hymenoscyphus fraxineus* within *Fraxinus* species and cultivars**  
J.A. HIEMSTRA, P. COPINI



11:50 – 12:10; **presentation 40**; **Situation and perspectives of ash (*Fraxinus* spp.) in Ukraine: focus on eastern border**

K. DAVYDENKO, V. MESHKOVA

### 12:10 – 13:50: LUNCH BREAK INCL. POSTER PRESENTATIONS

#### SESSION 11. Chair: JEAN BERUBE

13:50 – 14:10; **presentation 41**; **New insights into Dutch Elm Disease: cell wall compositional, ecophysiological, vascular and nanomechanical assessments**  
J. ĐURKOVIČ, F. KAČÍK, M. MAMOŇOVÁ, R. LAGAŇA, I. ČAŇOVÁ, J. URBAN, J. KRAJŇÁKOVÁ

14:10 – 14:50; **presentation 42**; **LIFE+ ELMIAS project on managing wooded meadows of Gotland under the threat of Dutch Elm Disease and Ash Dieback**  
K. WÅGSTRÖM, G. OLESKOG

14:50 – 15:10; **presentation 43**; **Fighting Dutch Elm Disease on the Gotland Island. What have we learnt and what can we expect for the future?**

J. STENLID, A. MENKIS, R. VASAITIS, K. WÅGSTRÖM

### 15:10 – 15:40: COFFEE / TEA BREAK INCL. POSTER PRESENTATIONS

#### SESSION 12. Chair: ALEJANDRO SOLLA

15:40 – 16:00; **presentation 44**; **Dutch Elm Disease in Estonia**

L. JÜRISOO, R. DRENKHAN

16:00 – 16:20; **presentation 45**; **Dutch Elm Disease in Ukraine: history, current situation and perspectives**

I. MATSIAKH, V. KRAMARETS

16:20 – 16:40; **presentation 46**; **Dutch Elm Disease resistance in a Romanian trial**

D. CHIRA, D. TURCU, I. CÂNTAR, O. MERCE, N. CÂNTAR, I. CHISĂLIȚĂ, C. CIOCAN, F. CHIRA, F. BORLEA

16:40 – 17:00; **presentation 47**; **Genomic study of yeast-mycelium dimorphism and its possible contribution to parasitic fitness in the Dutch elm disease pathogen *Ophiostoma novo-ulmi***

L. BERNIER

17:00 – 17:20; **presentation 48**; The endophytic fungus *Rhodotorula*-P5 induces a priming effect in *Ulmus minor* promoting the protection against *Ophiostoma novo-ulmi*

J. SOBRINO-PLATA, S. ORMEÑO, I. FERNÁNDEZ, C.M.J. PIETERSE, C. COLLADA, L. GIL, J.A. MARTÍN

17:20 – end

## **19:00: CONFERENCE DINNER**

**August 31<sup>st</sup>**

**SESSION 13. Chair: IBEN THOMSEN**

09:00 – 09:20; **presentation 49**; CRISPR-Cas9 strategies for editing candidate genes in the Dutch Elm Pisease pathogen *Ophiostoma novo-ulmi*

P. TANGUAY

09:20 – 09:40; **presentation 50**; Susceptibility of different ash species to Emerald Ash Borer (*Agrilus planipennis*) (based on the work done in the ash collection of the Main Botanical Garden in Moscow)

L. SERAYA

09:40 – 10:00; **presentation 51**; Emerald Ash Borer (*Agrilus planipennis*) in European Russia: history of the invasion and current situation

Y. BARANCHIKOV

10:00 – 10:20; **presentation 52**; Testing ash for resistance to dieback: an amateurish approach

A. MENKIS, K. DAVYDENKO, A. ZALUMA, J. STENLID, R. VASAITIS

**10:20 – 10:50: COFFEE / TEA BREAK INCL. POSTER PRESENTATIONS**

**SESSION 14. Chairs: JAN STENLID & ALEJANDRO SOLLA**

10:50 – 11:50; **Discussion**: What is the future for elms in Europe?

11:50 – 12:00; **boarding busses at Wisby Strand Conference**

12:00 – ... **excursion (program enclosed at the end of the book)**

## POSTER PRESENTATIONS IN UPPSALA

**53. Pathogenicity and development of *Heterobasidion annosum* and *Heterobasidion parviporum* in functional sapwood of middle-aged coniferous and broadleaved trees in Latvia**

A. ZALUMA, N. BURNEVIČA, T. GAITNIEKS, R. VASAITIS

**54. Evaluation of the occurrence of biological control agent Rotstop and native strains of *P. gigantea* in commercially treated stumps one to three years after treatment**

A. ZAŁUMA, K. KENIGSVALDE, A. VALDĒNA, T. GAITNIEKS

**55. Effect of silver and copper nanoparticles on the control of damping-off disease and growth in Scots pine (*Pinus sylvestris* L.) in the nursery of Spychowo Forest District, Poland**

M. ALEKSANDROWICZ-TRZCIŃSKA, A. SZANIAWSKI, A. SOŁTYS, M. STUDNICKI

**56. The influence of stress factors on the susceptibility of different tree species to *Heterobasidion annosum***

M. BARANOWSKA, H. KWAŚNA, P. ŁAKOMY

**57. The tree-fungus beetles collected onto the sawdust substrate with the mycelium of selected fungi species in the forests of the Experimental Forest Station in Rogów (Central Poland)**

A. BYK, J. PIĘTKA, J. BOROWSKI

**58. Diplodia tip blight in Germany: ecological and molecular characteristics of the tree pathosystem**

K. BLUMENSTEIN, J. BUßKAMP, G. J. LANGER, E. TERHONEN

**59. Wood decay of coniferous species caused by the mycelium of *Neolentinus adhaerens* (Alb. & Schwein.) Redhead & Ginns under laboratory conditions**

J. PIĘTKA, A. BYK

**60. Distribution of *Heterobasidion* and *Armillaria* root rot fungi in Vallombrosa fir forest, Italy**

L.B. DÁLYA, P. CAPRETTI, L. GHELARDINI, L. JANKOVSKÝ

**61. Diversity of invertebrate in *Heterobasidion* spp. fruit bodies on decayed spruce wood**

L. LEGZDINA, V. SPUNGIS, T. GAITNIEKS

**62. The effect of silver and copper nanoparticles on the growth and mycorrhizal colonisation of Scots pine (*Pinus sylvestris* L.) in a container nursery experiment**

M. ALEKSANDROWICZ-TRZCIŃSKA, A. SZANIAWSKI, M. STUDNICKI, M. BEDERSKA-BŁASZCZYK, A. URBAN

**63. Situation of *Armillaria* spp. and *Heterobasidion* spp. in Slovenia**

A. BRGLEZ, N. OGRIS

**64. Effect of injuries on stability of Norway spruce**  
G. SNEPSTS, O. KRISANS, E. DUBROVSKIS, R. KAPOSTINS, A. JANSONS

**65. Practical solutions for the surveillance and the containment of the invasive forest pathogen *Heterobasidion irregulare* in Italy**  
L. GIORDANO, F. SILLO, G. LIONE, P. GONTHIER

**66. Survey and Relative Abundance of *Heterobasidion* species distribution in Coastal British Columbia's Forests**  
S.F. SHAMOUN, G. SUMAMPONG

**67. Monitoring of *Phlebiopsis gigantea* on commercially treated spruce and pine stumps in Latvia**  
L. BRŪNA, A. VALDĒNA, A. ZAĻUMA, L.D. LUKSTIŅA, K. KENIGSVALDE, A. KENIGSVALDE, T. GAITNIEKS

**68. Development of *Phlebiopsis gigantea* fruit bodies on large diameter log pieces of Norway spruce and Scots pine**  
A. ZAĻUMA, L. BRŪNA, L.D. LUKSTIŅA, A. VALDĒNA, K. KORHONEN, K. KENIGSVALDE, A. KENIGSVALDE, T. GAITNIEKS

**69. Management of *Armillaria* using combinations of biological controls and plant defence activators**  
L.E. HAILEY, R.W. JACKSON, G.C. PERCIVAL

**70. Identification of candidate genes associated with the resistance QTLs in Norway spruce through a combined analysis of transcriptomics and genetic maps for *Pinaceae***  
R. CHAUDHARY, K. LUNDÉN, K. DALMAN, M. DUBEY, M.N. GORRIZ, B. KARLSSON, J. STENLID, M. ELFSTRAND

## **POSTER PRESENTATIONS IN VISBY**

**71. Biocontrol Potential of Forest Tree Endophytes**  
E. TERHONEN, A. KOVALCHUK, A. ZARSAV, F.O. ASIEGBU

**72. The microbial diversity and structure in peatland forest in Indonesia**  
B. LIU, M. J.H. TALUKDER, M. LAMPELA, H. VANSANDERB, E. TERHONEN, H. SUN, F.O. ASIEGBU

**73. Hormonal profiling of resistant and susceptible interactions between *Castanea* spp. and *Phytophthora cinnamomi***  
A. CAMISON, M.A. MARTIN, F. ALCAIDE, M. GONZALEZ, P. SANTAMARGARITA, P. SANCHEZ-BEL, V. FLORS, D. MORCUENDE, P. ABAD-CAMPOS, B. CUENCA, A. SOLLA

**74. Resilience of *Castanea sativa* to stress factors related to global change**  
F.J. DORADO, M.A. MARTIN, N. CHAVES, J.C. ALIAS, S. RODRIGO, B. CUENCA, E. CUBERA, A. SOLLA

- 75. Xylem anatomy of offspring of two *Ulmus minor* trees tolerant to *Ophiostoma novo-ulmi***  
J.A. MARTIN, A. SOLLA, A. ARROYO-GARCIA, A. RODRIGUEZ-GARCIA, L. GIL
- 76. Chestnut habitat restoration via the biological control of *Cryphonectria parasitica* in protected areas of Tismana (Romania)**  
D.CHIRA, C.MANTALE, F.CHIRA, M. PARASCHIV
- 77. Infection of *Fraxinus excelsior* by *Hymenoscyphus fraxineus* in pure and mixed young stands in Latvia**  
I. MATISONE, E. BADERS, L. PURINA, I. DESAINE, A. JANSONS
- 78. The presence of *Rhodotorula* sp. in *Ulmus minor* plantlets moderates the response to *Ophiostoma novo-ulmi* in roots of tolerant clones**  
J. SOBRINO-PLATA, C. COLLADA, C.M.J. PIETERSE, L. GIL, J.A. MARTÍN
- 79. Control of oxidative stress as a tolerance mechanism in *Ulmus minor* against *Ophiostoma novo-ulmi***  
J.A. MARTÍN, J. SOBRINO-PLATA, B. COIRA, D. MEDEL, C. COLLADA, L. GIL
- 80. The LIFE+ ELM project “elms alive” for the restoration of *Ulmus minor* and *Ulmus laevis* in Spain**  
J.A. MARTÍN, T. DOMÍNGUEZ-FLORES, J. DOMÍNGUEZ, J.I. GARCÍA-VIÑAS, I. GONZÁLEZ-DONCEL, G. GONZÁLEZ-GORDALIZA, D. LÉON, C. MAGRO-HERNÁNDEZ, C. MARTÍNEZ-ARIAS, D. MEDEL, E. MIRANDA, O. MIRAVALLS, J. RODRÍGUEZ-CALCERRADA, E. ZAFRA, L. GIL
- 81. Ash Dieback and Dutch Elm Disease: current situation and prospects in Slovenia**  
N. OGRIS
- 82. Physiological response and resilience to flooding of five *Ulmus minor* genotypes tolerant to Dutch elm disease**  
C. MARTINEZ-ARIAS, J. SOBRINO-PLATA, S. ORMEÑO-MONCALVILLO, N.M. AGUIRRE, D. MEDEL, L. GIL, J.A. MARTÍN, J. RODRÍGUEZ-CALCERRADA
- 83. The assessment of inoculum potential of *Armillaria gallica* in oak stands**  
T. RAŻNY, H. KWAŚNA, P. ŁAKOMY
- 84. Analysis of genetic and phenotypic diversity provide insights on the epidemiology of *Laetiporus sulphureus* in Sicilian carob trees**  
F. SILLO, L. GIORDANO, C. GIANCHINO, M. MARI, P. GONTHIER

# ABSTRACTS FOR TALK PRESENTATIONS IN UPPSALA

## SESSION 1

### 1. Species, distribution and spore density of *Heterobasidion* in Canada

J.A. Bérubé<sup>1\*</sup>, A. Potvin<sup>1</sup>, D. Stewart<sup>1</sup>, P.N. Gagné<sup>1</sup>, J.P. Ponchart<sup>2</sup>, J. Phelan<sup>3</sup>, A. Varga<sup>3</sup>, D. James<sup>3</sup>, E.D. Tremblay<sup>4</sup>, M.O. Duceppe<sup>4</sup>, T. Kimoto<sup>5</sup>, G.J. Bilodeau<sup>4</sup>

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*Heterobasidion irregulare* is a fungus causing a root disease capable of killing large trees. Infection probability is proportional to the density of its aerial basidiospores infecting freshly cut red pine stumps. We quantified for the first time *H. irregulare* aerial basidiospore density in southern Quebec and deposition rate along a southern Quebec transect. Spore counts from automated rotary arm spore collectors were determined using a ribosomal ITS TaqMan real-time PCR detection assay. Cumulative spore deposits on a 30 cm stump were estimated to be highest in the infected plantation with 2.01 spores per stump, 0.46 spore at a 500 m distance and 0.19 at 5km. On the transect study, total count was 706.3 spores in Harrington, rapidly decreasing with an eastward direction on the transect. With few exceptions, weekly spore depositions at all sites other than Harrington were greatly below 0.2 per m<sup>-2</sup> h<sup>-1</sup>. In a metagenomic study using Ion Torrent sequencing of aerial spores revealed presence of *H. annosum* s.s., in 7 samples from the provinces of Quebec and Ontario. It also showed presence of *H. abietinum*/*H. parviporum* DNA reads in soil samples and insect traps from British Columbia. A similar study using Illumina sequencing from local and imported ornamental nursery plants samples from British Columbia revealed the presence of *Heterobasidion irregulare* and *H. occidentale* DNA, *H. irregulare* having been mentioned only once before as present in BC. Surprisingly *H. irregulare* comprising 86% of the *Heterobasidion* DNA reads and more commonly found (13/15 samples) than *H. occidentale* (10/15 samples). These results will be discussed in a North American context.

## 2. Exploration of genetic and silvicultural approaches to protect Norway spruce from root rot caused by *Heterobasidion annosum sensu lato*

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The main research objective of this project is to investigate novel genetic and silvicultural approaches to protect Norway spruce from the *Heterobasidion* species. In continuous-cover silviculture the *Heterobasidion* infection spreads from tree to tree through root contacts and eventually causes rot in conifer forests. Therefore, mixed forests, such as, beech-spruce forests could increase the resistance of individual conifer tree to this fungal pathogen. To study this hypothesis, we test with inoculation experiments whether European beech is truly less susceptible against this root rot pathogen and could actually reduce the infections in the field. Inoculation experiments are currently on-going and results will be presented at IUFRO meeting.

We intend to test whether European beech is less susceptible than Norway spruce to these root rot pathogens. To have molecular proof of that, our next aim is to compare the defense related gene expression profiles of less susceptible European beech and the more susceptible Norway spruce activated during *Heterobasidion* infection. We expect to identify differences in the induction of genes related to signal perception and defense responses. The novel RNA-seq data will provide an important insight into transcriptional responses of less susceptible broadleaf tree and more susceptible conifer tree to *Heterobasidion* infection. Additionally, field inventories will be conducted to study if planting European beech as a mixed tree species reduces Norway spruce infection by *Heterobasidion*. The effect of beech-spruce mixed silviculture on Norway spruce health will be studied in monocultures and mixed forests in upcoming years.

**Keywords:** Forest protection, *Heterobasidion* root rot, mixed forest, European beech

### 3. Precision forestry for improved resource utilization and reduced wood decay in Norwegian forests

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Norwegian forest owners, foresters, and forest industry recognize *Heterobasidion*-caused wood decay of Norway spruce as a major problem that will be even more challenging in the future due to climate warming. The general opinion is that the current management practice is not sustainable. As a solution, we aim to develop a precision forestry framework for reducing the impact of root and butt rot while increasing overall sector value creation, improving resource utilization, and enhancing the economic robustness of the Norwegian forest sector. Precision forestry is defined as the planning and conducting of site-specific forest management activities and operations, and makes use of high technology sensing and analytical tools to support site-specific decision making. Here we 1) describe the magnitude of decay in Norway spruce forests in Norway, 2) present data from a survey registering the standpoint of Norwegian forest owners and foresters when it comes to the prevalence and control of wood decay, 3) outline our approach to develop precision forestry to combat wood decay, and 4) discuss some *Heterobasidion* spread biology related issues for which further research is warranted to improve disease modelling and implementation of precision forestry.

**Keywords:** automated harvester-assisted large-scale data collection, climate change, control



#### 4. Quo vadis, *Heterobasidion*?

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In the Czech Republic the root-rot fungus *H. annosum* (Fr.) Bref. became a problem ever since forest management had been introduced. It has been mainly *Heterobasidion annosum* which has been causing issues in forests on former agricultural lands and/or in secondary spruce stands. During the 19<sup>th</sup> and 20<sup>th</sup> century, the natural stands consisting of broadleaved trees (beech 40%, oak 20%, silver fir 20%) and minority conifers were changed into stands with conifers in majority (nowadays - Norway spruce 50%, Scots pine 20%). These interventions started after great floods during the end of 19<sup>th</sup> century and with hunger for energy source after world wars. Above all, the main reasons were economical purposes. Consequences of the unpremeditated treatment have started to be visible when global climate change resulted in higher temperatures and droughts came on. The ability of stands to withstand different biotic and abiotic factors have been naturally tested during last decade, resulting in clear cuts (salvage felling) in great scale which has never been registered during history of forest management. In 2017, 4.774 thou. m<sup>3</sup> were felled as salvage felling, besides in 2010 2.934 thou. m<sup>3</sup> were registered and felled as salvage felling. Massive cuttings resulted in a change of the main ideas of the tree species composition in new planted areas where broadleaved or mixed stands will be preferred. As the droughts and therefore damages on conifer stands are assumed to continue, the afforestation with spruce monocultures or forest monocultures in general will not be possible and therefore will be massively lowered. According to other data, between years 1961-1990 spruce was suitable to grow on 23.4%, during years 1991-2004 it was lowered to 10.4% and models predict lowering this number to 5.1 % and 3.6 % during years 2021-2040, 2041-206 respectively. In the Czech Republic, the importance of oak stands will be rising as a result, but also lands with no tree cover will be rising as the climate is predicted to be too arid for any tree vegetation. In this matter also importance of *Heterobasidion sp.* as the significant pathogen in forest system of the Czech Republic is assumed to be decreasing. Although, still a lot of plantations were established on hazardous lands within the last 20 years.

## SESSION 2

### **5. An inoculation experiment suggests that presence of the native forest pathogen *H. annosum* in European forests may facilitate the invasion by the exotic *H. irregulare***

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Since its introduction in 1944, the North American fungal forest pathogen *Heterobasidion irregulare* has become invasive in pine stands of central Italy and has broadly hybridized with the native congeneric species *H. annosum*. Several main drivers of this biological invasion have been identified, however little is known about the outcomes of direct interactions between the two species. In this study, by genotyping *Heterobasidion* fruiting bodies and mycelia developed in pine logs inoculated with both fungal species, we tested whether the coexistence of *H. irregulare* and *H. annosum* genotypes in the same portions of logs may: a) affect fruiting bodies production of either species; and, b) generate hybrids with specific nuclear-mitochondrial combinations. Results showed that *H. irregulare* developed a number of fruiting bodies 1.9-fold significantly larger when spatially overlapping with *H. annosum* than when by itself. However, all *H. irregulare* fruiting bodies were morphologically similar, independent of where they were formed. Notwithstanding that all possible nuclear-mitochondrial combinations were identified in hybrids, hybrids with nuclei of both species and the *H. irregulare* mitochondrion were favored, while hybrids with both nuclei of one species and mitochondria of the other species were less frequent. Additionally, on the log side where *H. annosum* genotypes had been inoculated, 33% of hybrid fruiting bodies had an *H. irregulare* mitochondrion, suggesting that the *H. irregulare* mitochondrion is favored compared to the *H. annosum* one. Based on these results, we predict that if the exotic fungus were to be introduced in central and northern European forests characterized by an incidence of *H. annosum* higher than that of central Italian forests, such introduction would result in a much faster spread of the invasive species, and in large hybrid swarms leading to abundant introgression of the *H. irregulare* mitochondrion into native *H. annosum* populations. Thus, we recommend surveys aimed at the early detection of *H. irregulare* should be performed using both nuclear and mitochondrial markers, and should be intensified in those European forests where the presence of *H. annosum* is relevant.

**Acknowledgement.** This work was supported by the Italian Ministry of Education, University and Research, within the PRIN program (grant number 2008SBCC9S)

## 6. Assessing the association between fitness of *Heterobasidion irregulare* × *H. annosum* hybrids and the mitochondrial genome

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The North American root rot agent *Heterobasidion irregulare* Garbel. & Orosina was accidentally introduced in central Italy in 1944, where the Eurasian congener *H. annosum* (Fr.) Bref. is also present. *H. irregulare* has become invasive colonizing pine and oak stands along 105 km of coastline west of Rome, and is currently recommended for regulation by the European and Mediterranean Plant Protection Organisation (EPPO). In search of an explanation for the invasiveness of the introduced species, several comparative studies have been published contrasting the biology and the epidemiology of the non-native *H. irregulare* and of the native *H. annosum*. These studies have investigated factors such as the pathogenic, saprobic, and sporulation potentials of the two species. However, the two species have also started to mate in Italy, and are currently admixing their nuclear and mitochondrial genomes. By assessing the pathogenicity, saprobic ability, and gene expression of *H. irregulare* × *H. annosum* hybrids and of their parental genotypes, we show that mitochondria influence significantly the saprobic growth of hybrids. Moreover, gene expression data suggest that fungal fitness is modulated by an intimate interplay between nuclear genes and mitochondrial type, and is dependent on the specific mito-nuclear combination.

**Acknowledgement.** This work was supported by the Italian Ministry of Education, University and Research, within the FIRB program (grant number RBFR1280NN)

## 7. Genomic admixing between *Heterobasidion irregulare* and *H. annosum* varies depending on the direction of interspecific introgression and may confer adaptive advantages to both species

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Hybridization between species is recognized as a major force leading to the rapid adaptive evolution of the species involved. However, how hybridization may shape the evolutionary trajectories of hybridizing species in fungi is still poorly understood. Large scale genomic admixing between the introduced North American pine pathogen *Heterobasidion irregulare* and its Eurasian sister species *H. annosum* is currently occurring in Italy, providing a unique opportunity to study the genomics of hybridization. In this study, we performed a comparative genomic analysis of natural *H. irregulare* x *H. annosum* hybrids with the aim of identifying what gene categories are recurrently introgressed from one species into the other. Whole genomes of nine natural homokaryotic hybrids collected from the airspora in the Circeo National Park, in Italy, were sequenced, *de novo* assembled, and compared with the genomes of six pure genotypes of the two parental species. Phylogenomic and genetic structure analyses determined the extent and the quality of genomic admixing in hybrids. Analysis of nucleotide diversity coupled with Tajima's D test, and alignment of *de novo* assembled genomic scaffolds were performed to detect recombinant and conserved genomic regions in hybrids. Based on Fisher's exact test for Gene Ontology terms enrichments, *H. annosum* alleles recurrently introgressed into *H. irregulare* were mainly related to putative regulatory pathways. By contrast, *H. irregulare* alleles introgressed into *H. annosum* were associated with putative saprobic processes and secretory Golgi-related pathways. Therefore, the interspecific hybridization and gene introgression ongoing in Italy are expected to improve the transmission potential of the native *H. annosum* while improving the regulation of genes of the invasive *H. irregulare*. Regulation of gene expression may be a critical aspect of the adaptation of *H. irregulare* in novel environments, and a further key to its success as an invasive species in Europe.

**Acknowledgement.** This work was supported by the Italian Ministry of Education, University and Research, within the FIRB program (grant number RBFR1280NN)

## 8. A new North American *Heterobasidion* genome and the new provisional hybrid species *Heterobasidion irregularicis* from Alpine larch

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Hybridization is recognized as one of the major forces involved in the rapid evolution of plant pathogens. In a high elevation alpine larch stand in Montana, the dominant fungal pathogen *Heterobasidion occidentale* has hybridized with a locally rare Eastern North American population of *H. irregulare*. A sequence analysis of one mitochondrial and four nuclear loci from several *Heterobasidion* genotypes documented that hybridization has increased allelic diversity by generating novel alleles unreported in either parental species. Based on sequence data from the loci analyzed, we show that all heterokaryotic genotypes with admixed nuclear genomes possess one *H. occidentale* nucleus, a *H. occidentale* mitochondrion, and a nucleus derived from the parental Eastern *H. irregulare* genome, but clearly distinct from it based on phylogenetic, structure, and PCoA analyses. Tajima's test shows that only *H. irregulare*-like alleles have undergone an accelerated evolution compared to alleles of other species within the *H. annosum* complex. We hypothesized that this rapid evolution may have been the result of hybridization followed by repeated backcrosses between hybrids and *H. occidentale* individuals only, due to the rarity of *H. irregulare* at high elevations. Spore trapping confirmed *H. occidentale* is clearly dominant in the Alpine larch stand in question, and results of ABBA/BABA tests were consistent with a hybrid origin of *H. irregulare*-like alleles, which we show contain genic blocks belonging to both species. Conversely, *H. occidentale* alleles found in hybrids, although novel, do not contain *H. irregulare* genic blocks and cannot be differentiated from alleles of "pure" *H. occidentale* individuals. All isolates from larch were heterokaryons comprising a *H. occidentale* genome, a new hybrid *H. irregulare* x *H. occidentale* genome, and a *H. occidentale* mitochondrion: we propose the name *H. irregularicis* to identify these chimeric heterokaryons found on Alpine larch.

### SESSION 3

#### **9. *Heterobasidion annosum* root rot in noble fir combined with bark beetle attack**

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Noble fir (*Abies procera*) is among the most susceptible *Abies* species in relation to *Heterobasidion* root rot. Stump removal is a necessity if the fungus is present on sites intended for noble fir and Fraser fir (*A. fraseri*). First generation agricultural land is preferable for establishment of high value crops such as noble fir Christmas trees and greenery (bow) production. However, *H. annosum* may gain entry via stumps of shelter belts and crop trees, if trees are not felled in period with frost, and preventive stump treatment is omitted.

In 2013 problems with *Cryphalus piceae* bark beetles were discovered in several noble fir greenery plantations in Denmark. This species had not been recorded in Denmark before, only the closely related *C. asperatus* which is considered harmless. In Denmark, fatal bark beetle attacks killing whole trees occur in stands affected by *Heterobasidion annosum* sensu stricto, as a result of *C. piceae* breeding in the bark of infected noble firs age 10-30 (5-10 meters tall). In one site the presence of both root rot and bark beetles was clearly associated with the removal of conifer shelter belts without use of stump protection against *H. annosum*. While *H. annosum* is certainly capable of killing noble fir by itself, the presence of the bark beetles accelerates the rate of tree mortality. This may in turn affect the spread rate of the pathogen in the stand, by faster growth in the root systems of dying trees. So far, the same association has not been observed in other fir species in Denmark such as *Abies alba* and *A. nordmanniana*, which may be due to the fact that both European silver fir and Nordmann fir are relatively resistant to *H. annosum*, and *H. abietinum* is not present in Denmark.



A. Noble fir killed by a combination of *Heterobasidion annosum* root rot and *Cryphalus piceae* bark beetles. B. *Heterobasidion* fruitbodies at base of young noble fir. C. *Heterobasidion* decay in noble fir in greenery stand. Photos: Mathias Just Justesen (A, C), Iben M. Thomsen (B).

## 10. Perspectives of growing *P. contorta* in Latvia: focus on *Heterobasidion* root rot

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In Latvia, the first experimental plantations of *Pinus contorta* Douglas ex Loudon were established almost 40 years ago. The first introductions were done due to resistance against needle cast as well as there were need for alternative tree species than Scots pine for plantations on pure, sandy soils for diversification purposes. Evaluation of growth in 30 year old pine plantations showed that the standing volume of *P. contorta* was about 25 % higher than in stands of *Pinus sylvestris* L. (240 m<sup>3</sup> and 170 m<sup>3</sup>, respectively).

The obtained results indicate that the exotic *P. contorta* has potential for use in practical silviculture in the Baltic region for wood biomass production. Establishment of plantations for biomass production has been considered in the Baltic states, where large areas of abandoned agricultural land suitable to this purpose are available. However, it was observed that *P. contorta* suffer from root rot. The aim of this work was to investigate the infection and spread of *Heterobasidion* spp. in *P. contorta* plantations in former agricultural and forest soils in Latvia.

*Heterobasidion* infection was observed in six out of seven inspected plantations. In three of them dieback-symptomatic trees were subjected to fungal isolations. All isolates were field-mapped and subjected to somatic compatibility tests to detect spatial distribution of genotypes and rates of expansion. Occurrence of *Heterobasidion* infected *P. contorta* varied from 9 to 19%. Both *Heterobasidion annosum* (Fr.) Bref. and *Heterobasidion parviporum* Niemelä & Korhonen was able to attack growing *P. contorta* via root contacts and thinning *P. contorta* stumps via basidiospore infection. Isolation frequencies of the pathogen from infected trees were high, and yielded pure cultures from 93.2 to 100% of the samples subjected to isolations. In total, 97 genets were detected. The maximal length of territorial clones in each study varied, maximal length reached almost 40 m. In conclusion, *P. contorta* is highly susceptible to *Heterobasidion* root infection. In thinning's of *P. contorta*, stump treatment with agents that prevent primary infections by *Heterobasidion* should be considered.



## 11. Fungal community structure in asymptomatic and symptomatic Norway spruce trees naturally infected by the conifer pathogens *Heterobasidion* spp.

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Plant microbiome plays an important role in maintaining the host fitness. Despite a significant progress in our understanding of the plant microbiome achieved in the recent years, very little is known about the effect of plant pathogens on composition of microbial communities associated with trees. In this study, we analyzed the mycobiome of different anatomic parts of asymptomatic and symptomatic Norway spruce trees naturally infected by *Heterobasidion* spp. We also investigated the primary impact of the disease on the fungal communities, which are associated with Norway spruce trees. Our results demonstrate that the structure of fungal communities residing in the wood differed significantly among symptomatic and asymptomatic *Heterobasidion*-infected trees. However, no significant differences were found in the other anatomic regions of the trees. The results also show that not only each of individual tree tissues (wood, bark, needles and roots) harbors a unique fungal community, but also that symptomatic trees were more susceptible to coinfection by other wood-degrading fungi compared to the asymptomatic ones.

## 12. Ecology and distribution of *Heterobasidion* species in Serbia

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*Heterobasidion annosum* is economically one of the most important pathogens of the coniferous forests of Northern Hemisphere. Differences in damages and preferences to different conifer species have influenced researchers to begin to treat *H. annosum* as the species complex. In late 1990's *H. annosum* s.l. has been divided into 3 European *Heterobasidion* species which were connected to their hosts' Scots pine, Norway spruce and European fir and named by Niemelä & Korhonen as *H. annosum*, *H. parviporum* and *H. abietinum*, respectively.

Study of root rot pathogens has a long tradition in Serbia and starts in the mid of 20<sup>th</sup> century when first epidemics of tree decline were connected with presence and spread of *Fomes annosus* (later *Heterobasidion annosum*) in a different area of Yugoslavia. In that time, there was no information about different species, but it was reported that damages in Scots pine and Norway spruce stands have differed. Root rot pathogens are biological component forest and as opportunists can use possibility and colonize stressed trees. Monitoring the presence of *Heterobasidion* spp. was done since species separation and information from earlier studies was reassessed and new species identification (both classical and molecular) performed. *Heterobasidion* species diversity, distribution, presence and spread of genets and chaining of the damages (e.g. bark beetle outbreaks, tree decline caused by abiotic factors) in last two decades will be presented on studied sites throughout Serbia.

**Keywords:** *Heterobasidion annosum*, root rot, tree decline, genets, Norway spruce, Scots pine.

## SESSION 4

### **13. Potential “new” protective agents for biocontrol of *Heterobasidion* spp. on Norway spruce**

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Root rot caused by *Heterobasidion* spp. is one of the most spread and devastating conifer diseases in the Northern Hemisphere. The spread by spores can be prevented by treating stumps with *Phlebiopsis gigantea*. In Sweden this is the only commercially available alternative with proven effect. There is therefore an increasing interest from the forestry sector in new biological agents and means of control for *Heterobasidion* spp. More consistent and effective genotypes of *P. gigantea* isolates would benefit the forest industry as well as reduce ecological risks connected with using just one isolate for treatment of conifer stumps locally. Therefore the aim of this study was to examine new biological protection agents on Norway spruce wood that could potentially be used commercially for stump protection against *Heterobasidion* spp.

The competitive ability against *H. parviporum* of ten different isolates of *P. gigantea*, including the commercially available Rotstop®S isolate and the biological treatment Proradix®, containing *Pseudomonas* spp. bacteria, were tested in laboratory conditions on wooden blocks infested with *H. parviporum*. In addition a small field study comparing Proradix® and Rotstop®S was conducted.

Results of the current study indicated that *P. gigantea* isolate S2384\_2\_VI was superior ( $p < 0,05$ ) to the other 9 isolates by means of average growth rate per day on top of wood heavily infected by *H. parviporum*. Rotstop®S together with the PG342 isolate had the fastest growth in the first two days of juxtaposition with *H. parviporum*. Wood colonized by *Pseudomonas* spp. bacteria *in vitro* is resistant to *H. parviporum* even after 25 days of close contact.

There are consequently possibilities to further develop the treatment of stumps using *P. gigantea*. *Pseudomonas* spp. bacteria also showed remarkable results in resistance of *H. parviporum in vitro* and demonstrated a potential ability to protect also stumps from primary infection with *Heterobasidion* spp. when compared to other treatment methods, yet it was only a pilot study of small scale. Therefore treatment with *Pseudomonas* spp. bacteria should be monitored in long term experiments before it can be considered as a potential alternative to *P. gigantea* in terms of biological stump control.

## 14. Development of *Heterobasidion* genets in even-aged Norway spruce stands on peat soils

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Root rot fungi *Heterobasidion* spp. cause great economical losses in managed conifer stands. In Latvia, Norway spruce (*Picea abies*) is one of the most important commercial tree species covering 19 % of the forest land. The aim of the study was to determine *Heterobasidion* spp. frequency in spruce stands on drained peat soils. The study focuses on three aspects: i) to determine *Heterobasidion* species causing root and stem rot ii) to analyse *Heterobasidion* spp. population structure and iii) to assess *Heterobasidion* spp. distribution in context of stand age.

The study was conducted in six previously inspected even-aged, 47 to 117-year-old spruce stands in four districts of Latvia. In all, 11 circular sample plots with an area ranging from 100 to 500 m<sup>2</sup> were established for detailed analysis of *Heterobasidion* spp. genets. In each plot, all trees and stumps were mapped and sampled for *Heterobasidion* infection. To assess distribution of decay in spruces of different age, 60 decayed trees were cut. Data from previous studies were added to calculate yield losses caused by *Heterobasidion* spp.

Out of 979 studied spruces 16.3 % were infected by *Heterobasidion* spp; 82 % by *H. parviporum* and 18 %, by *H. annosum*. In some stands up to 30% trees were infected by *Heterobasidion* spp. Out of the 71 *Heterobasidion* genets identified 75 % included only one tree or stump. One average, a single *Heterobasidion* genet contained 1.7 infected trees/stumps. The result reveals that on the studied peat soils the great majority of spruces suffering from *Heterobasidion* root rot were infected primarily by *Heterobasidion* spores. However, 25% of the primary infections had led to the spread of root rot to other trees through the mycelial growth. Yield losses caused by *Heterobasidion* spp. proved to increase by the age of the stand.

## 15. *Armillaria gallica* – distribution and DNA sequence variability

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*Armillaria gallica* Marxm. & Romagn. is an European opportunistic pathogen causing decline of various tree species –broadleaved and conifers. According to the taxonomic authorities Marxmüller and Romagnesi, the older names associated with this fungus were rejected (Marxmüller 1987): *A. bulbosa* (Barla) Kile & Watling is treated as a synonym of *A. cepistipes* Velenovský and *A. lutea* Gillet is treated as a dubious name of yellow forms of various species. Due to results of laboratory mating tests, *A. gallica* has been treated as circumboreal species due to dikaryon formation between isolates from Europe and North America. Nevertheless, recent molecular phylogenetic studies based on DNA sequences of translation elongation factor 1-alpha revealed the *A. gallica* isolates from North America and East Asia belong probably to several different species. Moreover some isolates from South or East Europe (Serbia, Ukraine) seem to differ in DNA sequences from those of typical European *A. gallica*. Therefore, a detailed population genomic analysis is needed to reveal relationship among different lineages of *A. gallica*.

## 16. Spatial distribution of wood decay fungi in Southern Moravian forest types

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Several wood rotting fungi contribute to the healthy functioning of forest ecosystems, whereas others cause infectious diseases and high economic losses. The occurrence of two important pathogens, *Heterobasidion annosum* s.l. and *Armillaria* spp. was investigated in natural and managed forests of the Dražanská vrchovina Highlands (Southern Moravia, Czech Republic). Besides, the presence of a range of other fungal taxa known as saprophytes/weak parasites was examined in decayed wood. Identification of different fungal species from soil, wood and fungal samples was accomplished by DNA-based methods (nested PCR, RFLP analysis, multiplex PCR, sequencing of the translation elongation factor 1-alpha gene). The influence of environmental factors on the presence/absence of the distinguished species was tested using binomial logistic regression. *H. annosum* s.s. and *H. abietinum* were mostly associated with conifer stumps. The DNA of *Armillaria* spp. were detected in all sampling plots. The most common species were *A. gallica* and *A. cepistipes*. *A. ostoyae* was absent in Natural Reserves. *Stereum* spp. and *Fomitopsis pinicola* were also found in a diverse spectrum of host trees and habitats.

## SESSION 5

### **17. A small secreted protein from necrotrophic pathogen *Heterobasidion parviporum* (HpSSP) induces cell death in *Nicotiana benthamiana* and elicits defense responses in Norway spruce**

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*Heterobasidion parviporum* Niemelä & Korhonen, the causal agent of root and butt rot, is the most damaging pathogen of Norway spruce (*Picea abies* (L.) Karst.) in Europe. In *Heterobasidion*-conifer pathosystem, the fungus is believed to deploy a repertoire of small secreted proteins (SSP), including known effector-like proteins, to promote the infection of the host and sustain the disease development. The published genome sequence for *H. parviporum* makes it accessible to identify the putative SSPs encoding genes. In our study, we identified a novel SSP encoding gene (*HpSSP35.8*) from *H. parviporum*, showing high similarity to *HaSSP30* in *H. annosum*. It encodes a 177 amino-acid protein with a putative signal peptide of 20 amino acids. We expressed *HpSSP35.8* in *N. benthamiana* by agroinfiltration and found that *HpSSP35.8* could induce a rapid and strong cell death on *N. benthamiana* leaves. *WRKY12* and *ERF1a* were induced in the interaction between *HpSSP35.8* and *N. benthamiana*. We also investigated the expression dynamics of *HpSSP35.8* and Norway spruce defense response in the interaction between *H. parviporum* and seedling roots. The results showed that *HpSSP35.8* was highly expressed at early stages. Transcription factors (*ERF1a*, *ERF1b* and *WRKY12*), that regulate pathogenesis related (PR)-genes, were found to be upregulated. A particular chitinase gene (*PR4*) was highly expressed during the infection. All these results indicate that *HpSSP35.8* is an important virulence factor for *H. parviporum*.

## 18. Physiological and molecular features of necrosis response in *Heterobasidion*-conifer pathosystem

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Norway spruce is the main softwood species utilized in the pulp and paper industry in Finland. However, every year the root and butt rot of conifer trees caused by *Heterobasidion annosum* leads to a huge economic loss not only in Finland but also in Europe. Once challenged with pathogens, a series of defense responses will be initiated in conifer trees. Among them, necrosis response is featured by the rapid and localized cell death, also known as hypersensitive cell death in crop plants. Differently from crop plants, in conifer trees, necrotic cell death does not stop the invasive growth of necrotrophic pathogens like *Heterobasidion*. But it can trigger other defense reactions in a relatively short time. Currently, defense mechanisms of conifer trees have been the subject of much research, but a combination of different developmental stages has been rarely reported. Besides, another important and common response in mature woody tissues called reaction zone has been comparatively seldom studied. To investigate this, Norway spruce plants of different developmental stages were inoculated with *Heterobasidion parviporum* and six indicators were assayed. We found strong necrosis (or increased lesion size), increased pH, higher peroxidase activity and induction of some defense genes in the infected seedlings, young trees and mature woody trees compared to respective uninoculated or wounded control. Similar colour changes after reaction zone dye application were observed in the sapwood comparable to observation in trees with heartwood. In conclusion, immature developmental stages of conifer trees could be good resource materials for resistance research.



## 19. Bacterial biota and correlation with host terpenoid profiles of asymptomatic and symptomatic Norway spruce infected by *Heterobasidion* sp.

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Forest trees live in close association with microbial symbionts, which may affect the host fitness, productivity and tolerance against biotic and abiotic stressors. The composition of forest tree microbial communities could be influenced by tree genotypes and micro-environment of the anatomical tissues, but little is known about the effect of tree pathogens on the structure of these communities. In this study, we investigated the structure of bacterial communities associated with different anatomical tissues of asymptomatic and symptomatic *Heterobasidion* rotten Norway spruce [*Picea abies* (L.) Karst.] trees. Our results demonstrated that each of the investigated tissues (root, bark, down stem, upper stem and needles) harbored a unique bacterial assemblage. However, the health status of the host trees had little effect on the structure of bacterial communities, as the only significant differences among asymptomatic and symptomatic trees were found in the composition of the bacterial communities of needles. Additionally, we performed profiling of terpenoid compounds present in spruce xylem and phloem. Total concentrations of monoterpenes and sesquiterpenes were considerably higher in asymptomatic trees. Our results provide an important insight into the diversity of bacteria associated with Norway spruce trees. This is the first comprehensive study on bacteria biota on conifer tree tissues. We hope the datasets will be a huge resource for the tree biology and forest pathology community as well as plant science community.

## 20. Metabolomics and RNA-seq provides novel insights into interactions between Norway spruce and necrotrophic pathogen *Heterobasidion annosum* s.l.

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Members of *Heterobasidion annosum* species complex are causative agents of root and butt rot of conifer trees. In order to get better understanding of interactions between *Heterobasidion* fungi and their hosts and of the mechanisms underlying tree resistance to root and butt rot, we performed dual RNA-seq analysis of host and pathogen transcriptome in naturally infected Norway spruce trees. We also analyzed phenolic and terpenoid contents of the spruce trees. Our data provide support for the contribution of the components of flavonoid pathway to the resistance of Norway spruce against *Heterobasidion* infection. At the same time, the accumulation of lignans was observed in trees with decay symptoms. We also identified a set of candidate genes, which might be involved in the higher level regulation of defense responses in spruce trees and found indications for a possible role of abscisic acid (ABA) signaling in this process. Fungal genes upregulated during the host colonization included carbohydrate- and lignin-degrading enzymes, secondary metabolism genes and several effector-like genes. Results of this study contribute to better understanding of defense strategies employed by conifer trees against *Heterobasidion* infection as well as potential pathogenicity factors deployed by the fungus for necrotrophic growth. Identified host candidates deserve further evaluation as potential markers for higher resistance against root and butt rot.

## SESSION 6

### 21. Root and stem rots of forest trees in Central Siberia

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During the last 12-30 years intensive group dying of conifers has been observed in southern forest of Siberia and Russian Far East. Main tree species subjected to the decline are *Abies sibirica*, *A. nephrolepis*, *Pinus sylvestris*, *P. sibirica*, *P. koraiensis* and *Picea obovata*. Mortality is mostly due to the activation of root rot fungi *Armillaria* spp., *Heterobasidion annosum* s.s., *H. parviporum*, *H. abietinum* and locally also *Phellinus sulphurascens* and *Phaeolus schweinitzii*.

In the forests of Siberia and Russian Far East we found *Armillaria* spp.: *A. borealis*, *A. ostoyae*, *A. sinapina*, *A. calvescens*, *A. gallica*, *A. cepistipes*. *A. borealis* is a common and aggressive root pathogen in southern taiga of central Siberia, killing frequently pine, spruce and fir, even trees showing no signs of decreased vitality, especially on sites with shallow soil on hard rock. Main reason for the activation of root pathogens seems to be the climate change: increase in temperature, precipitation and wind load, combined with local edaphic factors. Cutting of forests, anthropogenic pollution and insects are additional promoting factors.

Root rot caused by *H. annosum* is particularly common in pine stands growing on soil with loam interlayer among sandy deposits; this creates favourable moisture condition for the fungus. Pine decline does not extend beyond the limits of edaphic anomalies. *H. parviporum*, *H. abietinum* and *P. sulphurascens* cause root and butt rot and mortality on spruce and fir. Because the attacks of root rot fungi are largely dependent on edaphic factors the mosaic structure of soil should be taken into consideration at the time of reforestation.

Trees weakened by root rot are more susceptible to insects and other diseases (e.g. *Neonectria fuckeliana* for *A. sibirica*). A new phenomenon is to decrease of the aggressive of *Armillaria* in the last two years. Further research on the long-term sites allow to understand the causes of these dynamics.

Among the fungi that cause stem rots are widespread in Central Siberia: *Fomitopsis officinalis*, *F. pinicola*, *Ganoderma applanatum*, *G. lucidum*, *Porodaedalea pini*, *Laetiporus sulphureus*, *Fomes fomentarius*, *Piptoporus betulinus*, *Phellinus tremulae*, *P. igniarius*.

**Keywords:** climate change, edaphic factors, resistance, root rot disease

## 22. Resistance breeding against *Heterobasidion* spp. in Norway spruce

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This project is focused on the root rot fungus *Heterobasidion annosum* s.l., a major pathogen of forest trees, and its interactions with the hosts Norway spruce and Scots pine. Root rot of conifers is economically very devastating. The *Heterobasidion* spruce pathosystem in Sweden costs approx 500-1000 million per year in downclassed timber, reduced tree growth and mortality of trees. We have identified thirteen quantitative trait loci (QTL) that are associated with lower spread of the fungus in Norway spruce. One of the loci contains a gene encoding for leucoanthocyanidin reductase (LAR), an enzyme active in catechin biosynthesis. The association of one allelic variant with higher expression of LAR in more resistant trees has been confirmed in several independent spruce genetic materials. Work is now in progress to characterize genes from within the other spruce QTL regions. By including forest breeders in the research team we aim at implementing resistance characters in practical forestry. Marker assisted selection can be implemented in e.g. seed orchards, selection of parents in controlled crossings, or in choice of somatic embryo lines. Lack of correlation between resistance and growth parameters in the host as well as a minimal selection pressure for increased virulence in the pathogen speak in favour of long term sustainability of breeding efforts. We have also developed a dense set of markers based on exome capture technology and both genotyped and phenotyped major components of the Swedish spruce breeding program as well as the seed orchard trees in both northern and southern Swedish seed orchards. The results will be deployed in robust selective seed harvests and in future breeding programs.

### 23. Transgenerational defence priming in *Pinus pinaster*

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Transgenerational priming can be used by trees in order to cope with biotic and abiotic stressors. In this research, we tested whether seedlings originating from mechanically and hormonally stressed mother trees would be primed against biotic attacks. Mother trees were subjected to mechanical wounding, clipping and Methyl-Jasmonate (MeJa) spraying. Three full-sib families were obtained by controlled pollination and subjected to MeJa, *Hylobius abietis* (pest) and *Heterobasidion annosum* (pathogen) bioassays. Gene expression was explored by RNAseq and candidate genes were confirmed by qPCR. Mechanical wounding and MeJa treatment in mother trees triggered a higher relative expression of genes involved in terpene biosynthesis and in the phenylpropanoid pathway following MeJa application in the offspring. Differential regulation of histone related genes in offspring coming from MeJa treated mothers was found at constitutive level. Bioassays with both *H. annosum* and *H. abietis* showed different patterns when comparing seedlings from stressed/unstressed environments. Mother trees subjected to mechanical wounding showed a larger tolerance to *H. annosum* infection than those coming from control mother trees. No differences were observed in the case of *H. abietis*. Our study showed that trees are able to prime their offspring in order to adapt to biotic stressors, particularly to pathogens. Transgenerational priming can contribute to forest resilience against stress.

## **24. Genome-wide DNA methylation analysis provides insights on regulation of necro-/saprotroph lifestyles and developmental stages in *Heterobasidion parviporum***

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*Heterobasidion parviporum* is the causative agent of root and butt rot disease of Norway spruce. Conidia and mycelia are two important developmental stages in the asexual life cycle of this species. This pathogen also has a dual nutritional lifestyles (necrotrophy and saprotrophy). A major feature of this species is that it can flexibly switch lifestyles depending on the substrate. Nothing is however known on how the switch between the lifestyles are regulated. The hypothesis for this project is that the lifestyle transition and fungal development are governed by differential expression of genes, which are further regulated by DNA methylation. DNA methylation is one of the main epigenetic modifications in eukaryotic genome. In fungi, methylation is more frequently found in transposable elements (TEs), where it is thought to play a role in preventing the duplication of TEs and silencing of DNA repeats. The primary aims of this project are to profile the genome-wide distribution of methylation in *H. parviporum* and to determine the implications of DNA methylation in the development and different lifestyle stages in this species. Total RNA and genomic DNA were extracted from conidiospores and free living mycelia of *H. parviporum* as well as from necrotroph and saprotrophic stages and used for RNA-seq and Bisulfite sequencing, respectively. Bioinformatic analysis revealed that methylation occurred in any DNA context including both CpG and non-CpG contexts, but with preference for CpG context. The distribution of mC sites was not even, but displayed a strong, preferential methylation in TEs. Analysis of differentially methylated loci indicated that necrotrophic and saprotrophic lifestyles might share highly similar pattern of gene usage. When combined with RNA-seq data, the expression of some genes involved in signal transduction such as those encoding kinase-like domains, G-protein beta WD40 repeat and RNA polymerase might be constrained by DNA methylation in response to different lifestyles and developmental stage other than free living mycelia.

## SESSION 7

### **25. Resistance of Scots pine trees to *Heterobasidion annosum* infection: gene expression profiling and terpenoid analysis**

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In the forest of Northern hemisphere, a considerable percentage of conifer trees cut are rotted and commercially less valuable, largely due to infection by a single pathogen *Heterobasidion annosum* s.l. The combined direct and indirect economic effects of *H. annosum* infection approximate to 50 - 100 million euros alone in both countries and about 0.8 billion euros in losses to European forestry yearly. Although the pathogen could be managed by silvicultural methods or pretreatment of stumps with biocontrol agents or chemicals, there is no 100% percent protection. Modern biotechnology offers the opportunity to search and identify other novel molecular, genetic or chemical fingerprints relevant for host resistance. In this project, twenty two 10-15 year-old Scots pine trees from at Lapinjärvi, Uusimaa-Finland were inoculated to determine their response to *H. annosum* infection under field condition. Seven months post inoculation, the necrotic lesion size were documented. The expression patterns of genes encoding terpenoids known to be involved in tree defences were assayed on sample harvested prior to the infection. Similarly, chemical analysis of diverse range of monoterpenes and sesquiterpenes were also analysed. Increased transcript abundance of genes encoding 3-carene 1 synthase (PbTPS-3car2), caryophyllene/humulene synthase (PsTPS6), and RNSI1 synthase (PaRNSI1) was documented in highly tolerant genotypes. Equally,  $\alpha$ -thujene and Trans- $\beta$ -ocimene accumulated in highly tolerant genotypes. The potential of these molecules to serves as a biochemical marker for tree selection and breeding programs will be discussed

## 26. Disease control of *Phytophthora plurivora* and other *Phytophthora* species infecting European beech and rhododendron in the United States

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*Phytophthora plurivora* is a root rot, canker, and foliar pathogen that is thought to have been spread worldwide by the nursery industry. The pathogen may be native to southeast Asia, where it is found in the soil and rhizosphere of apparently healthy trees in the forests of China, Nepal, and Taiwan. However, in Europe, *P. plurivora* is associated with root rot of forest tree species and causes blight of rhododendron and other ornamentals in nurseries. In the United States (U.S.), *P. plurivora* is one of several *Phytophthora* species causing canker on mature, landscape European beech trees and is an important foliar and root rot pathogen in nurseries on ornamental nursery crops, including rhododendron. Recent nursery surveys (2013-2017) have shown that *P. plurivora* and *P. cinnamomi* are the most common species causing root rot of rhododendron in the northwest U.S. Yet, despite decades of research, *Phytophthora* root rot remains a significant problem in the nursery industry. A better understanding of the diversity of *Phytophthora* species involved and how that diversity affects disease control is needed to improve disease control efficacy and thereby minimize the spread of invasive pathogens in the nursery industry. Here, we report on fungicide treatments that were successfully used to treat cankers on European beech trees and on our recent efforts to improve the efficacy of fungicide treatments for root rot of rhododendron. Differences in sensitivity to mefenoxam and phosphorous acid, the two most commonly used fungicides, were observed among *Phytophthora* species and isolates infecting both European beech and rhododendron. In addition, the method of fungicide application was critical for achieving disease control for both hosts: bark drenches were most effective for canker control on European beech while soil drenches were most effective for root rot control of rhododendron. Research is underway to determine whether differences in fungicide sensitivity among *Phytophthora* species and isolates affects disease control in rhododendron.



## 27. Screening *Quercus suber* and *Q. ilex* for resistance to drought and *Phytophthora cinnamomi*

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In Spain, current global change leads to an increased demand by foresters and land owners of cork and holm oak trees tolerant to drought and *Phytophthora cinnamomi* (*Pc*). In November 2015, acorns from 84 asymptomatic holm oak and 15 asymptomatic cork oak trees located in *Pc*-diseased areas of Extremadura were collected. The acorns were planted in root trainers according to a split-plot design and four experiments were carried out. (1) In June 2016 and 2017, 3,000 seedlings were inoculated with *Pc*. At the end of both vegetative periods the mortalities recorded for *Q. ilex* were 26 and 97%, respectively, higher than those observed in *Q. suber* (15 and 65%;  $P < 0.01$ ). Heritabilities of 'time to death' trait were  $0.57 \pm 0.02$  and  $0.69 \pm 0.04$  in *Q. ilex* and *Q. suber*, respectively. (2) Additional 3,000 seedlings, inoculated in June 2016 and 2017 with *Pc*, were subjected in May 2017 to severe water stress. Probably because of the synergy of water stress and *Pc* effects, final mortalities of *Q. ilex* and *Q. suber* plants were nearly 100%. Surprisingly, differences in susceptibility between species observed in (1) disappeared. (3) To determine tolerances to drought of progenies, additional plants were deprived of irrigation until death. No relationship was observed between tolerances to *Pc* and tolerances to water stress. (4) To confirm that results in the greenhouse matched in the field, in 2018 two plots with 18 seedlings per progeny and plot were installed in diseased areas. Moreover, 304 genotypes are being cloned to be part of the material selected for registration and commercialization.

## 28. Offspring of ink-diseased chestnut trees in response to water stress and *Phytophthora cinnamomi*

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Most stands of *Castanea sativa* in Europe are infected by *Phytophthora* spp., responsible of 'ink disease'. It is hypothesized that 'ink disease' influences the early performance and susceptibility to water stress and *P. cinnamomi* in chestnut offspring. In a common garden, the germination of chestnuts and the performance of seedlings from non-infected and *P. cinnamomi*-infected *C. sativa* mother trees were assessed. Plants were further submitted to water stress and *P. cinnamomi* inoculations. To understand the basis of inherited maternal effects, gene expression analysis was conducted. Performance and tolerance to drought of seedlings were strongly influenced by the health status of the mother trees, i.e. offspring of healthy trees was significantly taller and wilted more than offspring of 'ink diseased' trees ( $P < 0.001$ ). Mortality due to *P. cinnamomi* was similar in both groups of offspring. However, when dividing offspring according to plant height, the group of plants 1-10 cm tall from 'ink diseased' trees died significantly less and slower than plants 1-10 cm tall from healthy trees. Gene expression was not helpful in explaining results, and further studies would require considering plant size as a factor that can influence defense response of offspring. Increased tolerance of offspring of 'ink diseased' chestnuts to water stress suggests that seeds were primed for water stress tolerance during maturation. Increased tolerance to *P. cinnamomi* of short seedlings suggests a growth-defense trade-off occurring only in offspring of 'ink diseased' chestnut trees. Ecological consequences of transgenerational phenotypic plasticity in offspring of forest trees will be discussed.

## SESSION 8

### **29. Beech bark disease in Slovakia: history and current situation**

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Although, beech bark disease in Slovakia does not cause a major problem at present, it caused extensive local damage in the past. However, considering to dominant presence of beech in Slovak forest stands, there is still a great risk of wood raw material losses. In this short review, we summarized past and current knowledge about beech bark disease in Slovakia. The results of ongoing long-term disease monitoring of as well as the impact of environmental factors are presented. The article also provides a list of hitherto known *Nectria* sp. in relation to other host plants and their hazardousness and occurrence. But, above all, we wanted to point out to needfulness of histopathological research of beech tissues attacked by different types of nectriae. Defensive response mechanism at anatomical and biochemical levels of host cellular tissues as well as the strategy of decomposition by different pathogens have not been explored yet in beech. The mentioned facts and challenges are going to be solved in the newly designed project which is also presented.

**Keywords:** Beech bark disease, *Nectria* sp., histopathology, bark tissues, xylem tissues

**Acknowledgement.** This work was supported by the Slovak scientific grant agency VEGA No. 1/0149/15.

### 30. Emerald ash borer *Agrilus planipennis* Fairmaire, 1888 (Coleoptera: Buprestidae) within its native range in East Asia

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Even though the emerald ash borer, *Agrilus planipennis* causes huge economic, ecosystem and aesthetic damage within its invasive ranges in European Russia and North America, where it has killed hundreds of millions of ash trees since early 2000s, it is still just a minor pest within its native range in Asia. Only limited information is so far available on its biology and impact in natural and anthropogenic ecosystems within its native range.

**Native range.** Recently, so far the most comprehensive database containing 108 localities of museum and literature records of *A. planipennis* in Asia was compiled (Orlova-Bienkowskaja, Volkovitsh, 2018). It confirms that the emerald ash borer naturally occurs in Russian Far East (Primorskiy Kray and Khabarovskiy Kray), China, South Korea, and Japan; whereas records from Inner Mongolia and Sichuan (China), Mongolia, Taiwan, and Laos are considered ambiguous or proved incorrect. In Russian Far East, the species was first collected only in 1946 in the Sikhote-Alin Nature Reserve (Primorskiy Kray) and that record was published as late as in 1979 as *Agrilus* (*Anambus*) *markopoli* Obenberger, 1930 (Alexeev, 1979).

**Host plant.** Under natural conditions in East Asia, larvae of *A. planipennis* feed on native *Fraxinus mandshurica* Rupr., *F. chinensis* ssp. *rhyrachophylla* (Hance) A.E. Murray (including *F. japonica* Blume ex K. Koch as a synonym), and occasionally on *F. chinensis chinensis* Roxb. Within its native range, the beetle is also recorded on introduced and planted European ash (*F. excelsior* L.) and American ash species (*F. americana* L., *F. nigra* Marshall, *F. pennsylvanica* Marshall, *F. quadrangulata* Michx., *F. velutina* Torr.). In Japan and South Korea, *A. planipennis* has been also reported on *Juglans mandshurica* var. *sieboldiana* Maxim., *Pterocarya rhoifolia* Siebold & Zucc. (both Juglandaceae) and *Ulmus davidiana* var. *japonica* (Rehder) Nakai (Ulmaceae), however there is no evidence whether the species was reared or only collected on these Japanese plants (reviewed by Orlova-Bienkowskaja, Volkovitsh, 2018). In East Asia, the emerald ash borer mainly attacks weakened native ash species, whereas the impact is high and damage is more severe on introduced ash species which likely lack resistance to the beetle.

**Seasonal cycle.** Within its native range, the life cycle of *A. planipennis* was studied only in a few locations. Similarly to other buprestids, the life cycle of the emerald ash borer is rather flexible. Depending on the temperature conditions, the complete cycle can take two years with overwintering of young larvae and then prepupae (characteristic of cooler regions like Harbin and Changchung, China) or just one year with overwintering of prepupae (as in warmer regions like Tianjin, China). It is likely that in some region, populations can consist of individuals which develop two years

and those who managed to reach adulthood with a single year (reviewed by Orlova-Bienkowskaja, Bienkovski, 2016). The eco-physiological mechanism controlling voltinism of *A. planipennis* has not been studied.

**Perspectives.** To facilitate development of control programs of *A. planipennis* in Europe and North America, it might be important to study the following aspects of the pest within its native range in East Asia: (1) resistance mechanisms in Asian ash species (first of all, Chinese ash *F. chinensis* and Manchurian ash *F. mandshurica*) and hybrids between Asian and European or North-American ash species, (2) selection of resistant forms and hybrids of ash species, (3) natural control agents (first of all parasitoids), (4) synergetic effect of *A. planipennis* and the ash dieback fungus *Hymenoscyphus fraxineus* on ash species.

**Acknowledgement.** The work was supported by the Russian Foundation for Fundamental Research (grant 17-04-01486).

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### 31. *Tomostethus nigritus* sawfly damage on ash in Finland

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*Tomostethus nigritus* (Fabricius) sawfly is classified in Finland vulnerable (VU) species (Viitasaari & Paukkunen 2010), which habitats on ash are estimated to be strongly fragmented and dispersed. In 2013 the Forest Damage Diagnostic Service of Finnish Forest Research Institute was informed for the first time that the species was defoliating ashes in Tuomarinkylä, Helsinki, close to the nursery shop yard (Heino & Pouttu 2014). At the same time, the first *T. nigritus* damage on ashes was observed in Scotland, Aberdeen (Stockan & Taylor 2014).

*T. nigritus* appears to be gradually losing the status vulnerable in Finland. In summer 2017 there were many observations of the species. The first foliar damage were recorded in Lohja, southwestern Finland, on 3.7.2017. Larvae were found in the trees. Weak symptoms of *Hymenoscyphus fraxineus* were found in the same area too. More damage was detected in July. In Järvenpää, southern Finland, three completely defoliated around 20 meter tall ashes (only petioles and seeds were left) were found on 11.7.2017. Later it appeared that there was extensive defoliation in several surrounding smaller trees and in some seedlings too. No larvae were detected, but empty larval skins were found; larvae may have descended in the soil. Two other announcements were received of ash defoliation in the Helsinki region also. All the damage of 2017 were not confirmed to have been caused by *T. nigritus* because no adult insects were found. However, *T. nigritus* feeds mainly on leaf edges, and this way of feeding was detected in all these cases.

*T. nigritus* overwinters as co- or pronymph in soil and pupates early in spring. The risk of damage during the next growing season may be estimated by soil samples in autumn. Spring control should be carried out in May-June when larvae are still small. Tens of swarming adult insects were detected in mid-May 2018 in the Järvenpää monitoring area, and species identification was confirmed. The eggs were hatching after two weeks, and the first foliar damage on the ash trees was found at the end of May. A more comprehensive monitoring of the ash damage situation was made in 18 sites, both ash stands and park trees, in Uusimaa, southern Finland in July 2018. No new damage areas were detected, but the ash defoliation was much more large-scale and intensive, and the feeding began earlier both in Lohja and in Järvenpää in the growing season of 2018 than in that of 2017. Some ashes have died in Lohja, probably due to the extensive defoliation, but in Järvenpää it appeared that the trees growing on soils with high ground water level recovered well, even from very extensive feeding, during two growing seasons.

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### 32. *Scolytus multistriatus* on Gotland Island: phenology and infectiousness with Dutch Elm Disease

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*Scolytus multistriatus*, the smaller European elm bark beetle, is a vector for Dutch elm disease (DED) that in year 2005 invaded the island of Gotland. The island possesses large and highly valuable population of elm (mainly *Ulmus minor*) in northern Europe. In this study we have monitored flying activity of *S. multistriatus* during 2012-2018 using pheromone traps. In addition, the beetles that were sampled during 2012-2014 were assessed for communities of vectored fungi using high-throughput sequencing. Sampling of the beetles was carried out at two different sites in Gotland. In total, 50 traps were placed at each site and checked weekly during June-August each year. From all sites and years, 335 beetles were trapped. Among these, 27.1% were trapped in June, 55.9% in July and 17.0% in August. Sequencing of ITS rDNA from the beetles revealed the presence of 1589 fungal taxa, among which virulent DED pathogen *Ophiostoma novo-ulmi* was the second most common species (9.0% of all fungal sequences). *O. ulmi*, the less virulent DED pathogen, was also detected but only in a single beetle sampled in 2012 (0.04% of sequences). There were 13.0% of the beetles infested with *O. novo-ulmi* in 2012, 4.0% in 2013 and 27.7% in 2014. The results demonstrated that proportion of *S. multistriatus* infested with *O. novo-ulmi* may sharply change in a single year. In order to limit the spread and damage by DED, control measures including harvesting and destruction of diseased elms should be completed before the intense flying period of the beetles.

**Keywords:** *Ophiostoma*, invasive pathogens, bark beetles, disease management, fungal community, *Ulmus*



# **ABSTRACTS FOR TALK PRESENTATIONS IN VISBY**

## **SESSION 9**

### **33. Veteranisation – using tools instead of time**

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The number of ancient trees has declined dramatically over the last few hundred years primarily due to agricultural intensification and forestry. Fewer trees are allowed to go through the natural aging process to reach the very great ages they can achieve and pollarding has often been abandoned. Other issues affecting existing ancient trees include climate change, tree diseases, ill-advised management and competition from surrounding younger trees. Many of the existing ancient tree sites across northern Europe are isolated and vulnerable, often with very few remaining ancient trees and with a large age gap between the old trees and their successors. Currently the only way we know for trees to develop habitat associated with ancient trees and their associated biodiversity is to wait. It is only after oaks for example, reach an age of 200 to 300 years, that hollows begin to develop.

Veteranisation has in fact been around for centuries in the form of pollarding for example. Today, however we use the term to describe a technique whereby younger trees are “managed” in a way which may speed up the process of development of valuable habitats, found otherwise only on very old trees. Such habitats may range from hollows to single dead branches or exposed wood. The idea is to try and mimic nature using manual tools.

Not everyone subscribes to the idea of damaging young trees for nature conservation gain. Here we describe why veteranisation is worth considering, where it may be appropriate and some different techniques that may be tried. In addition some of the early results from an international trial that was set up in 2012 with 20 sites and 980 oak trees, to evaluate the impact of veteranisation on a more scientific basis (Bengtsson et al, 2013; Hedin et al 2018), will be presented as well as ideas for the next steps.

### 34. Predicting ash dieback severity and environmental suitability for the disease in forest stands in the Czech Republic

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Ash dieback, caused by the ascomycete fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya, has been rapidly expanding across Europe during the last two decades, posing a considerable threat to native ash populations. Many aspects of ash dieback have been intensively studied and many new findings in disease epidemiology have emerged, which resulted in development of advisable silvicultural strategies and guidelines for sustainable management of ash species under *H. fraxineus* invasion at the local as well as international level. However, suitable predictive models and maps of pathogen distribution and impacts on forests and landscapes at the local level have not yet been developed in countries with high disease impact. Even though, the identification of locations at the highest as well as the lowest risk of invasion and damage is crucial for the implementation of effective management strategies to control invasion and protect ash stands at the state level. We aimed to develop spatial predictions of the current ash dieback severity and the environmental suitability for the disease across the geographic distribution of ash in forests of the Czech Republic.

Species distribution models in conjunction with geographic information systems have been increasingly used in forest pathology to relate the geographic distribution of pathogens and diseases to factors that vary across landscapes, create suitability maps, predict the spatial patterns of diseases and prioritize locations for early detection, monitoring and control of disease outbreaks. Our regression-based models are based on an extensive field survey carried out at 1,169 locations distributed throughout heterogeneous habitat conditions within the Czech Republic and relate the extent of ash dieback to host species availability, silvicultural characteristics, geomorphological, edaphic and climatic conditions of the locations, and characteristics of the neighbouring landscapes. A model of actual ash dieback severity relates disease extent to silvicultural and environmental characteristics of forest stands and their neighbourhood, while a model of environmental suitability for the disease quantifies the relative susceptibility of sites to the disease, independent of the current silvicultural characteristics. The developed models were subsequently used for interpolations to the remaining forest stands containing ash within the Czech Republic to produce final predictive maps.

Areas that face considerable risk of the invasion of ash dieback and severe damage to ash stands as well as the areas at the low risk of damage were identified. The maps suggested that fertile lowlands (e.g., along the Ohře River in northern and the Elbe River in eastern and central Bohemia, and along the Dyje and Morava Rivers in southern and central Moravian valleys) and humid areas bordering Poland and Slovakia (e.g., Moravian-Silesian Region and Beskydy Mts.) were the most endangered regions. These areas coincide with environmentally friendly conditions for the pathogen, i.e., high soil and/or air humidity and nutrient-rich soils, and have relatively high occurrence of ash trees in forests. Areas at the lowest risk of damage were concentrated predominantly in higher elevations of the Bohemian Massif in the frontier mountain ranges surrounding the country in the south, west and north (mainly the Šumava, Ore and Sudeten Mountains) and some inland highlands (e.g., Doubovské hory Mts., Central Bohemian Highlands, Křivoklát Highland) with less precipitation and usually poor soils on acid bedrock.

We believe that the developed predictive maps can help to implement effective management strategies to control the invasion of *H. fraxineus* and protect ash stands at the state level. Predictions of actual disease severity can serve as a useful tool for guiding the current management of infested stands whereas predicting environmental suitability is helpful for making long-term strategic decisions, e.g., identifying areas where future ash regeneration and cultivation may be unsuccessful.

**Keywords:** *Fraxinus excelsior*, *Hymenoscyphus fraxineus*; predictive modelling; fungal invasion; forest disease management; landscape pathology.

**Acknowledgements.** We thank the workers of the Forests of the Czech Republic state enterprise for collecting field data, M. Zavrťálek (Forests of the Czech Republic) for organizing the fieldwork, J. Valenta (Forests of the Czech Republic) and P. Kolařík (Forest Management Institute) for assistance in providing input data and V. Zýka (The Silva Tarouca Research Institute for Landscape and Ornamental Gardening) for help with GIS analyses. This work was supported by the [Ministry of Agriculture of the Czech Republic] under Grant [QJ1220218]; the [Czech Ministry of Education, Youth and Sports] under Grant [VUKOZ-IP-00027073]; and the [Technology Agency of the Czech Republic] under Grant [TH03030306].

## 35. Factors associated with the severity of ash dieback in south-western Germany

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Ash dieback is causing a strong decline of European ash (*Fraxinus excelsior*) in Europe. The agent of this tree disease is the fungus *Hymenoscyphus fraxineus* (anamorph: *Chalara fraxinea*). In the summer of 2015, data of the severity of crown symptoms and the occurrence of ash collar necroses were collected on a random subset of sample points of the national forest inventory (NFI). The data represents an area of about 35,000 km<sup>2</sup> in south-western Germany and comprises more than 1,600 ash trees (DBH > 7 cm) on 529 sample points at 330 tracts (grid points of the NFI). The sampling design allowed deriving proportions of tree health categories for different reference values, such as proportion of trees, occupying area or timber volume (standing stock). By analysing the ash dieback data together with other available information some factors were identified that are associated with the disease. The crown symptoms of the disease were more severe on trees that were dominated by others. Both crown symptoms and collar necroses were reduced on sites with smaller basal area of ash. Moreover, there were spatial differences in the extent of crown damage and collar necroses, which was less severe in the north-eastern part of the investigated area. Collar necroses occurred more often on trees of smaller DBH and of crown defoliation. They also were less frequent on sites with higher inclination. This and the spatial patterns of symptom occurrence suggest that collar necroses prevalence is influenced by soil moisture. The mean temperature and precipitation of the growing seasons of years 2006 to 2013 were not significantly connected to the prevalence of collar necroses, nor were the numbers of days with maximum temperatures exceeding 28 °C and 36 °C, respectively.

### Literature

Enderle, R., Metzler, B., Riemer, U. and Kändler, G. 2018. Ash Dieback on Sample Points of the National Forest Inventory in South-Western Germany. *Forests* 9(1), 25; doi: 10.3390/f9010025

## 36. Pragmatic decision-making for NATURA 2000 ashwoods in the face of Ash Dieback and other threats

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Climate change and novel pests and pathogens are already creating novel environmental conditions for woodlands, and this is likely to increase especially in the long term. Long term planning for conservation woodland has to take account of these changes, but our uncertainty over the nature and timing of environmental changes means we cannot be certain that any management approach will necessarily lead to the results we seek – the continued viability, distinctiveness and richness of the woodland type in question. This paper outlines an approach in progress to develop future management plans for ash-wych elm woodlands in Scotland to meet EU Natura requirements. The approach tackles the challenge in two ways – firstly by breaking down our uncertainty about future environmental conditions as far as we can into specific threats with estimated probabilities, and secondly by proposing different management approaches across different ash-wych elm woodlands to reflect the estimated likelihood of specific threats to the woodland system.

### Introduction

The Scottish examples of Tilio-Acerion forests of slopes, screes and ravines (EUNIS H9180) are limited in tree species due to their latitude and post-ice-age isolation. There is no native *Tilia* or *Acer*, and the canopies are dominated by ash *Fraxinus excelsior*, birch *Betula pendula*, Wych elm *Ulmus glabra*, oak *Quercus robur/petraea* and in the most oceanic locations, hazel *Corylus avellana*. Ash and Wych elm (and to some extent hazel) are the species dependent on richer soils, and thus are the characteristic and defining tree species of this woodland type. Ash in particular has characteristics that make its functions and supported species unique compared to other native trees in Scotland (Mitchell 2014), and the structural and shade-tolerant characteristics of Wych elm are similarly unreplicated by other species in these woodlands (Thomas 2018).

Scottish Natural Heritage has begun a review of the objectives and actions for H9180 woodlands as part of a wider review of Natura 2000 management in Scotland. This review is explicitly long term in scope, and indeed a review of any woodland type would be scarcely worthwhile without a long term perspective. Given that many woodland processes are slow – tree growth, ground flora and saprophyte colonisation for example, the concept of ‘long term’ for woodland is must surely be very long in contrast to much of human experience – decades and centuries rather than months and years.

Using that concept of ‘long term’ means that any review of habitat objectives and supporting actions inevitably must inevitably consider changes in the future environment of that habitat, especially climate change and novel biotic interactions (pests, diseases and altered competitive/complementary relationships between

species). H9180 woodlands are an interesting case for this work, because the characteristic and defining tree species - ash and wych elm – have current and potential serious tree health threats. The arrival in Scotland of Ash dieback since 2010 and the spread of Dutch elm disease over most of Scotland are clear, current and arguably existential threats to these woodlands, with the emerald ash borer as the most concerning known potential threat at present.

Long term planning for conservation woodland based on a rapidly changing environment is developing as we gain new appreciations of the scale, pace and substantial uncertainties of that change. The maintenance of functions or key characteristics in the face of new or changing external pressures is increasingly covered by the rapidly expanding literature on the topic of 'resilience'. From this literature we have taken the view that resilience is a context-dependent concept – i.e. to use it, we need to understand the objective (*resilience of what?*) and the threats (*resilience to what?*). We have thus developed an approach based on Fuller and Quine (2016) and Iason (2017) which we've termed **site-level resilience planning**. This paper outlines our approach and describes some key issues and likely practical management implications.

### **Site-level Resilience planning methodology**

Based on the foundation of **resilience of what, to what**, both Iason (2017) and Fuller and Quine (2016) produced similar proposals for developing resilience actions for specific ecosystems or habitats. The elements of our process adapted from this work are described below:

a) **Select a small number of system-critical elements** (= *resilience of what?*). An ecosystem is hugely complex, and developing resilience actions for every aspect or species would be an intractable problem. Choosing system critical species or processes allows us to concentrate on what really matters (Ellison 2005). An initial discussion has suggested the following as system-critical elements for H9180 (to be confirmed through our project):

- Semi-continuous low level of grazing
- Absence of damaging invasive species e.g. Rhododendron ponticum
- Continuous presence of mature and large trees with the characteristics of ash
- Continuous presence of mature and large trees with the characteristics of Wych elm

b) **Assess threats to the viability of those system-critical elements** (= *resilience to what?*). Future threat assessment is hard, especially when the typically long timescales of natural ecosystems are considered. In particular the continuous presence of mature trees - say 200 years old - requires us to consider threats that might arise in that time frame. For example, while uncertainty might be high, it is necessary to consider high-impact threats that might occur in 100 years' time. Put another way, planning on the basis that these woodlands would only face threats for the predictable future of the next 20 years or so is, in effect, an assumption that which no further threats would develop further into the future. Such an assumption seems rather heroic and unlikely to have any evidence to support it. Methodologies for classifying and comparing risks are likely to be helpful (e.g. the IUCN Red List

Threat Impact Score system (<http://www.iucnredlist.org/technical-documents/classification-schemes/threats-classification-scheme>).

c) **Develop actions to adequately protect the system-critical elements from the anticipated threats.** Actions that provide adequate protection for system-critical elements might include species or genotype changes, long term surveillance, preventative systems (e.g. fire preparedness, biosecurity), research or technical development or landscape-scale changes. Some actions coming from this process might be controversial, transformative, expensive or likely to challenge current approaches, and some might be unacceptable to key stakeholders. At this stage it is important to complete the analysis on the basis of effectiveness, without too much pre-judging of what might be acceptable or feasible.

d) **Implement, monitor and review** The number of potential actions deriving from (*system-critical elements x threats*) is potentially large, and will require further refinement to develop a practical set of options for stakeholders. It seems likely that there will be actions that should be implemented very widely, and some that might apply only on sites with specific attributes – or only apply off-site entirely. A key part of this process is that it provides us with a means of combining actions for the present time which can be updated or adapted as new evidence or projection become available, or as new technology provides new capacities.

### **Deriving management actions from the threat analysis**

The process described above –assessing threats to the viability of system-critical elements, especially ash and Wych elm - will seek answers to some demanding questions, such as:

- For these tree species, what can we say about their future health status that we can use to develop operational decisions?
- What will be the mortality rate for ash dieback, and over what period?
- What can our interventions through tree breeding achieve?
- What will be the interactions between climate change and the implications of ash dieback in the future?
- Will Emerald Ash Borer colonise Scotland?
- Will DED continue to spread through Scotland?

To avoid taking decisions in a position of spurious confidence, it is important to ask these questions, even if high-probability answers are not available. In other words, if there are important things we don't know, we should integrate that absence of knowledge into our response and decision-making.

Linking the threat assessment (with all its attendant uncertainties) to the selection of protective or pre-emptive actions provides us with some help in accounting for those uncertainties in our management choices. Currently, our management objectives and prescriptions for Natura Tilio-Acerion forests in Scotland are identical across the whole range of this habitat, with a uniformity very common in conservation management. That uniformity stems from the period when we thought we knew what the future held for our habitats, and thus sought to optimise the condition of every site through a common management approach. Looking forward with much less certainty, we are proposing to develop a diversity of prescriptions to both reflect the estimated probability of different future tree health scenarios, and to avoid a uniform

vulnerability to any future threat – not putting all our eggs in one basket. Some examples of how that might work in practice:

- **EASY:** A current threat is persistent overgrazing damaging woodland structure and tree regeneration. This is very likely to be a future threat, and the necessary action is to implement measures to reduce grazing to low levels. Because the high probability of this threat, we're likely to recommend that action on overgrazing is taken on 100% of the H9180 sites.
- **DIFFICULT:** The probability of very high mortality of ash in Scotland in coming decades (ash dieback, EAB, ineffective remedies) requires assessment as part of our project. If we could be sure that the probability was 0.25, we might be justified in proposing actions in response on 25% of the H9180 sites, but we are probably going to be much less certain and our actual decision might be as fuzzy as actions on the sites comprising half of the total - to be revisited as further information becomes available.

## Conclusions

For conservation, especially forest conservation with its long timescales, the period of simple certainties (justified or not) has gone. With it has gone the confidence that a certain form of management will lead to certain outcomes in the long term. That must not mean a paralysis of decision-making - as Lindner (2014) puts it, *Decision makers in forest management have to realise that they must take long-lasting management decisions while uncertainty [about climate change impacts] is still large.*

The methodology we're proposing for our review of Scotland's Tilio-Acerion woodlands is an attempt to break down our uncertainty about the future into more useful components – at perhaps a minimum level of *likely, unlikely* and *somewhere in between* – which can then be used to develop management responses to be implemented in some proportion to the perceived risk.

Is this good enough? A clear weakness is that if our assessment of the likelihood of a particular threat is wildly wrong, then the implementation of protective responses will be out of proportion to the impact – perhaps too small or unreasonably widespread a response. Overall our approach feels less comfortable than the confidence with which forest conservationists (including myself) used to prescribe management actions, where we felt able to assert '*do X, get Y*'. But the alternative to openly trying to reflect our uncertainty in our actions is surely to behave with spurious confidence and unstated assumptions that are based on unexamined uncertainty. For us as conservation managers committed to securing the future of these habitats, what other choice is there?

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## SESSION 10

### **37. Evaluation of the impact of *Hymenoscyphus fraxineus* in ash stands in Serbia**

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The ash dieback was for the first time reported in Serbia on *Fraxinus excelsior* and *F. angustifolia* in 2015. Since the first report, the pathogen spread to the eastern and south-eastern parts of Serbia towards borders with Bulgaria, Romania and FYR of Macedonia. The aim of the study was to evaluate the impact of the disease on the health condition of ash trees and regeneration in stands and plantations in Serbia. The field studies were carried out over the whole country of Serbia. Distribution maps of European ash (*Fraxinus excelsior*) and narrow-leaved ash (*F. angustifolia*) were made of the data obtained from the National Forest Inventory (2004–2006). Twenty-five publicly owned forest sites in which *Fraxinus* species occurs were selected in Serbia. The selection of studied sites was non-random; the aim was to select representative ash forest types in different regions of the state. Different symptoms of the disease are present on leaves, branches and stem, but basal cankers have not been observed on the stems of ash trees so far. The disease is present both on young and adult trees, but the damages are more serious on younger trees because of apical shoot loss and deformation of the stems. The regeneration process in all ash stands is threatened by the pathogen. Observed defoliation on adult trees is in the range of 5–35% and the percentage of dead trees does not exceed 6% for all evaluated trees/stands. Monitoring of established study plots has shown that the annual progress of the disease, in young plantations, is at the level of 7–9% for the period of 2016–2018.

**Keywords:** *Fraxinus excelsior*, ash dieback, *Chalara fraxinea*, defoliation, tree decline.

### 38. Consideration of traits that facilitate invasibility of *Hymenoscyphus fraxineus* in Europe

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Dieback of European ash (*Fraxinus excelsior* L.) is posing a continental-scale threat to this keystone tree species. The causative ascomycete *Hymenoscyphus fraxineus* is native to Asia, where it is a harmless leaf associate of local ash species. The dieback of European ash represents a showcase scenario of forest damage that can follow from exposure of evolutionary naïve trees to invasive alien pests that have a high level of adaptation to congeneric tree species in their native range. Besides the inherent host defence mechanisms, endophytic microorganisms have been shown to critically affect host physiology and performance, suggesting that evolution and ecology of plants can only be understood in a holobiont (host and its associated microorganisms) context. According to the stochastic niche theory of resource competition, invasion and community assembly, the probability that an invader could survive, reach maturity and reproduce would be highly dependent on its traits relative to the traits of the established species. The annual colonization of ash leaves is a crucial key dependency for the invasiveness of *H. fraxineus*, since its fruiting bodies are formed on overwintered leaf vein tissues in soil debris. Leaves of European ash host a wide range of indigenous epiphytes, endophytes, facultative parasites and biotrophic fungi, including *Hymenoscyphus albidus*, a relative of *H. fraxineus* that competes for the same sporulation niche as the invader. At face value, leaves of European ash are colonized by a large and diverse indigenous mycobiome. In order to understand why this invader became successful in Europe, we discuss and summarize the current knowledge of diversity, seasonal dynamics and traits of *H. fraxineus* and indigenous fungi associated with leaves of European ash.

**Keywords:** fungal life-styles, genomics, host specificity, reproduction modes

### 39. Variation in susceptibility to ash dieback caused by *Hymenoscyphus fraxineus* within *Fraxinus* species and cultivars

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Ash dieback caused by *Hymenoscyphus fraxineus* is increasingly causing dieback and death of ash trees in the Netherlands in forest plots and in urban green infrastructure. In this study we focus on ash trees used in urban green. In the Netherlands in urban green infrastructure *Fraxinus excelsior* and many of its cultivars are important, but also several other *Fraxinus* species and cultivars are commonly used. Although the disease is common in the urban area, large variation in severity were observed, possibly due to genetic variability within the genus. To determine the susceptibility of different *Fraxinus* species and cultivars 15 species (1 individual /species) and 24 cultivars were propagated on seedling rootstocks in a large scale field experiment with over 750 individual trees planted in 2016. Trees were inoculated by introducing a small wood chip infested by the pathogen between bark and wood on the stem (Kowalski, Holdenrieder 2009) in August 2017. Final examination of outward symptoms as well as discoloration within the wood of the stem was done in August 2018. This presentation will summarize the results of the above field screening of a large collection of ash species and cultivars and the results of some smaller experiments investigating the differential effects of different strains of the pathogen and the timing and development of symptom expression in inoculated trees. Finally, preliminary results on relating the results of the field screening to analysis of the tested collection using the molecular markers for tolerance of European ash (*Fraxinus excelsior*) as described by Harper *et al.* 2016 will be discussed. Outcomes of this study will provide information on disease risk of a large array of *Fraxinus* species and cultivars and on less susceptible alternatives for some susceptible genotypes common in Dutch urban green infrastructure. Moreover, validation of molecular markers for tolerance will strongly enhance future selection of ash genotypes more tolerant to ash dieback.

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**Acknowledgements.** The research leading to these results has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 634179 “Effective Management of Pests and Harmful Alien Species – Integrated Solutions” (EMPHASIS). The author thanks Jirka Rozsypálek and Michal Prouza (Mendel University, Brno) for their assistance in the field work.

#### 40. Situation and perspectives of ash (*Fraxinus* spp.) in Ukraine: focus on eastern border

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In Ukraine, the stands of common ash (*Fraxinus excelsior*) occupy only about 6% of all deciduous forests, however *F. excelsior* is important keystone species in parks, rural plantations, forest shelterbelts and taken together has considerable socio-economic importance. Since early 90s, dieback of European ash, caused by the alien invasive fungus *Hymenoscyphus fraxineus*, has spread over Europe including Ukraine where pathogen was isolated first in 2011 in the eastern region. There is concern about the conditions of ash forests in the central and western areas. Conditions of ash stands in Ukraine has worsened since 2006, when area of dead and drying up stands has exceeded 3.4 thousand ha. Damages caused by pests and pathogens with special emphasize on ash dieback were tested between different forest site types as well as the effects of relevant climatic factors on ash dieback from large-scale forest monitoring networks. Since 2006 index of forest health conditions for ash trees has tended to worsen (from 1.7 to 3.3 on average), while the most dramatic and fast ash decline was observed on the border with clear cutting (from 1.9 to 4.3 on average). Intensity of the ash dieback seems to be considerably higher at sites with high humidity where severe mortality of ash trees has been observed since 2010, associating with collar lesions frequently (73% of monitored ash trees in 2017) caused by *Armillaria* spp. The proportion of trees affected by *H. fraxineus* with collar lesions at dry sites was only 5.6% in 2017. Our study demonstrated that the proportion of ash trees with collar lesions greatly increased since 2012 in wet sites (in 3.8 times) to compare with ash at dry sites where ash sawfly damage was the highest and mixed infection showed an opposite pattern. The statistical analysis revealed clear differences between forest site types in the probability of pest and pathogens outbreak starting. Moreover, there appeared to be no significant relations between collar lesions occurrence and ash dieback at dry forest sites in the eastern region while the high correlation between ones was observed in the northern and central regions.

Therefore, ash dieback caused by mixed infection of *H. fraxineus* and *Armillaria* spp. has been observed at the humid forest sites mostly. Moreover, young infected or dead ash seedlings near the infected trees or stumps accurately indicated infection, but often seedlings remained uninfected around diseased tree at the dry forest sites. Therefore, last decades, *H. fraxineus* and *Armillaria* spp. played a crucial role in ash decline at humid forest sites while the ash sawflies and *H. fraxineus* caused decline of ash at the dry forest sites in the eastern region that significantly reduced ash stands area in Ukraine.

## SESSION 11

### **41. New insights into Dutch elm disease: cell wall compositional, ecophysiological, vascular and nanomechanical assessments**

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Comprehensive assessments were made of the chemical profiles of woody cell wall components, and also leaf growth, ecophysiological, vascular and nanomechanical traits for two Dutch elm hybrids 'Groeneveld' and 'Dodoens' which possess contrasting tolerances toward Dutch elm disease (DED). Upon infection with *Ophiostoma novo-ulmi* ssp. *americana* × *novo-ulmi*, medium-molecular weight macromolecules of cellulose were degraded in both hybrids. A loss of crystalline and non-crystalline cellulose regions occurred in parallel. In 'Groeneveld' plants, syringyl-rich lignin provided a far greater degree of protection from cellulose degradation, but only guaiacyl-rich lignin in 'Dodoens' plants was involved in a successful defence against the fungus. Unexpectedly, we found a very high proportion of non-significant differences between the infected and non-infected plants of 'Dodoens', including similarities in leaf growth, leaf gas exchange and leaf midrib vascular traits, as well as in the nanomechanical properties of the cell walls of tracheary elements such as modulus of elasticity, adhesion and energy dissipation. Three years after initial inoculations, except for a few traits such as leaf slenderness, relative chlorophyll content, transpiration rate of branches and sap flow density, we found no evidence of a decrease in leaf trait performances among the infected plants of 'Dodoens', despite the occasional persistence of fungal hyphae in the lumens of leaf midrib tracheary elements.

**Acknowledgements.** The authors thank Drs. M. Dvořák, R. Gebauer, R. Plichta, D. Olčák, M. Moravčík and T. Priwitzer for their contribution to the research on Dutch elm disease in Slovakia. This work was supported by the Slovak scientific grant agency VEGA No. 1/0149/15 (50%) and the Slovak Research and Development Agency under the contract No. APVV-16-0326 (50%).

## **42. ELMIAS: LIFE + project on managing wooded meadows of Gotland under the threat of Dutch Elm Disease and Ash Die-Back.**

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The Dutch Elm Disease (DED) and Ash DieBack (ADB) are today spread and established in more or less whole of Sweden. As a consequence, ash and elm were put on the Swedish redlist (threatened species) in 2010. On Gotland there has been ongoing combat against DED since it's discover in 2005. Until 2013 the fight against DED was sparse, but between 2013 and 2017 the combat was highly intensified and also successful in its combat of DED. The reason for this was the Life +- project, LifeELMIAS, partly financed from the European Commission. LifeELMIAS main activities, to remove and destruct infected trees, restoration measures to replace and minimize the loss of dead trees and information campaigns to reduce spread and new introductions of DED.

Since the introduction on Gotland of DED in 2005, infected elms have continuously been removed and destructed to minimize further spread of DED. It seems that today more than 90% of the elms (*Ulmus minor*) on Gotland have survived DED. To be compared with the other large Swedish Island, Öland, (west from Gotland) where only 5-10% of the elms have survived. The DED started to spread on Öland pretty much at the same time as on Gotland, in 2005, but on Öland no measures at all were taken to combat DED.

The reason to put resources on combat of DED on Gotland is because of high nature conservation values connected and dependent on elms, e.g. insects, lichens, birds, herbs, bats etc. These forested meadows with elms and ashes are quite unique in Europe when it comes to red listed species (threatened species) connected to elm and ash. By combat of DED, the loss of a huge number of species dependent on elm is limited. The situation is even more stressed because of the spread of ash die-back disease on Gotland as many red listed species are dependent also on ashes. From experience it's clear that it's extremely important to continuously combat DED without pauses. If there's no efforts taken to combat DED one year the disease will spread very fast and the costs to combat DED the coming year will be unnecessary high compared to a continuous combat. Most important, a pause will reduce the possibilities to combat DED at all.

Therefore it is important to continue the efforts to control DED on Gotland. The forested meadow habitats on Gotland are unique from a conservation- cultural-value point of view and valuable to preserve from a Swedish as well as European perspective. The combat against DED will continue till end of 2018 when LifeELMIAS ends. The cost for the combat of DED is estimated to 5 millions sek/year. These monies are needed to continue the fight against DED and for the future preserve elms and valuable conservation- and cultural- values dependent on elms on Gotland.

### **43. Fighting Dutch Elm Disease on the Gotland Island. What have we learnt and what can we expect for the future?**

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The elm forests and meadows at the Gotland Island in the Baltic Sea represent one of the last large elm resources in Europe. In 2005 an outbreak of Dutch Elm Disease (DED) was noted at Vallgård in central Gotland. The outbreak potentially threatened the whole elm population estimated at approximately 1 million trees together with the unique associated biological and cultural values of the meadows. The authorities decided early on to restrict the spread of the disease by monitoring disease symptoms during summer and felling and destructing trees in the winter season. The main target was the breeding material for the bark beetles that vector the disease. The disease control has been carried out yearly since 2007 supported by local governmental funds and a Life + EU project and some additional funds from the National Protection Agency and the Swedish Forest Agency. This has resulted in that approximately 5 % of the original elm tree population has been removed over a 13 year period. Interestingly, at the same time on the second largest Swedish Island in the Baltic Sea, Öland, a parallel outbreak of DED occurred starting also in 2005. In contrast to the situation on Gotland, on Öland no control measures have been taken and the host population has decreased to about 5 % of its original size. The presentation will discuss the efficacy of the control program and also project different management options in future scenarios. Discontinuations of the DED control at Gotland will very likely result in a loss of the majority of old large-sized elm individuals on the island within a period of 10 years with an associated loss of biological and cultural values of the Gotland meadows.



## SESSION 12

### 44. Dutch Elm Disease in Estonia

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DED had been registered in Estonia already in 1930-s (Lepik, 1940) as *Ophiostoma ulmi* (*Graphium ulmi*), but was reported in EPPO lists for Estonia only since 1979 (EPPO, 2017). Nowadays it is known, that the causative agent of DED is mainly species *O. novo-ulmi* Brasier, and it's two subspecies – *O. novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* (Brasier and Kirk, 2001). Since 2006, *O. novo-ulmi* had been identified also in Estonia, but not on the subspecies level (Hanso and Drenkhan, 2007).

Totally, 1225 elms were investigated at 4 different sampling sites and 2 sub-sites during 3 years (2014-2016) in Estonia, from urban space and countryside (incl. forests). For the first time, both subspecies of this invasive pathogen: *Ophiostoma novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* were detected. *O. novo-ulmi* subsp. *americana* inhabited only in northern Estonia, Tallinn, where elms had been suddenly declining since 2013 than in other sampling sites of Estonia. After 24 months a repetitive observation of 118 elms' health condition was done.

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## 45. Dutch Elm Disease in Ukraine: history, current situation and perspectives

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There are four *Ulmus* species are common in the forests of Ukraine: field elm (*U. minor* Mill.), Chinese elm (*U. parvifolia* Jacq.), Scotch elm (*U. glabra* Huds.) and European white elm (*U. laevis* Pall.). *Ulmus* species occupy more than 99,800 ha growing mainly in the forests of the State Forestry Agency of Ukraine and on 14,200 ha of those forest plantations *Ulmus* species can be found as 5 or more units. Dutch elm disease in Ukraine was first reported on the territory of Golovanetskie Forestry (Podillya) in 1929 (Shevchenko, 1986). The mass spread of the disease was observed in the 1960's and 70's of the XXth century, when the disease progressed to an epiphytotic level. In the 80's there was a decline in the activity of the Dutch elm disease (Shevchenko, 1987). The spread of disease and the cuttings in the forests led to the decrease of tree species age in the forests with elms. Nowadays, 41-60-year-old (55%) and 21-40-year-old (32%) elms trees are dominated in the forests and 0,2% of 100-year-old *Ulmus* species still can be found in the forests.

There is a chronic, at least acute, decline of elms of all ages around Ukraine. Since 2000, the foci of disease and affected *Ulmus* species have been recorded on the areas of 2,4-3,0 thousand ha.

The study related to Dutch elm disease in Western Ukraine has recently started in summer 2018. Sampling was done in two regions on Ukraine: Ternopil and Lviv. The main purposes of this studies are accurate identification of Dutch elm diseases pathogens and searching of resistant *Ulmus* trees.

#### 46. Dutch Elm Disease resistance in a Romanian trial

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European elms were severely affected by Dutch elm disease (DED) in several waves, due to the high proliferation of *Ophiostoma ulmi* and *O. novo-ulmi*. The invasive pathogens gradually killed elm trees from autochthonous species in all Romanian forest areas. A trial with elm populations from all over Romania was established in 1992, in “Pădurea verde” Timișoara (south-vest Romania). Local provenances of European elm species (*Ulmus glabra* Huds., *Ulmus minor* Mill., *Ulmus laevis* Pall.) were tested along with the Asian host (*Ulmus pumila* L.) of the fungi. Elm seedlings were artificial inoculated with *O. novo-ulmi* in 1993-1994. DED infections have led to a continuously mortality (the phenomenon is still active in 2018) among the tested materials. In 2018 the surviving materials consists of several resistant trees (with no sign of infection) of *U. glabra* and several tolerant trees (carrying infections but still having good development) of all European tested species. Siberian elm trees have been generally resistant. Local individual continuous tolerance to DED for 25 years offers high prospects for revitalisation of elms in Romanian forests.

**Keywords:** European elm species, artificial inoculation, *Ophiostoma novo-ulmi*, DED tolerance.

**47. Genomic study of yeast-mycelium dimorphism and its possible contribution to parasitic fitness in the Dutch elm disease pathogen *Ophiostoma novo-ulmi***

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The highly aggressive ascomycete fungus *Ophiostoma novo-ulmi* is responsible for the current, ongoing Dutch Elm Disease (DED) pandemic which has had a dramatic impact on populations of *Ulmus americana*, the most widespread native elm species in North America. *Ophiostoma novo-ulmi* displays yeast-mycelium dimorphism, a trait frequently encountered in fungal pathogens but which has received only limited attention in the DED fungi. The general objective of our current research is to identify molecular pathways underlying yeast-mycelium dimorphism and assess the contribution of this trait to parasitic fitness in *O. novo-ulmi*. Based on genome-wide RNAseq studies of *O. novo-ulmi* gene expression *in vitro* and *in planta*, we are targeting 1) genes in the Mitogen-Activated Protein Kinase (MAPK) and cyclic AMP-dependent Protein Kinase A (cAMP-PKA) pathways; 2) genes involved in nitrogen uptake and metabolism; 3) genes involved in utilisation of fatty acids and their transformation into derivatives including oxylipins; and 4) other genes that display high differential expression during the transition from yeast- to mycelial growth forms. Knockout and/or knockdown mutants have been obtained for some of the genes we have targeted and their effect on the *O. novo-ulmi* phenotype *in vitro* and *in planta* will be presented and discussed.

#### **48. The endophytic fungus *Rhodotorula*-P5 induces a priming effect in *Ulmus minor* promoting the protection against *Ophiostoma novo-ulmi***

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The antagonism of certain endophytes against the pathogenic fungus *Ophiostoma novo-ulmi*, causal agent of Dutch elm disease (DED), suggests they could be involved in tree tolerance mechanisms. To test this function, in a first trial plantlets of tolerant and susceptible elms to DED were inoculated with *O. novo-ulmi* and *Rhodotorula* sp. strain P5 (putative antagonistic endophyte) separately. In order to compare the plant responses to both, the endophyte and the pathogen, the presence of both fungi and the expression of 13 genes were quantified in the shoots of these plants. A typical defense response mediated by salicylic acid was observed in all genotypes with both fungi, regardless of their degree of tolerance. However, this response was greater with the pathogen inoculation. In a second trial, a third treatment was incorporated: the pre-inoculation with P5 one week before *O. novo-ulmi* inoculation. On this occasion, besides quantifying the presence of both fungi and analyzing the expression of the same genes, total phenols and flavonoids contents, and oxidative stress were measured. On the one hand, all genotypes showed an overexpression of different genes when they were inoculated with the pathogen, among them- were those that code for pathogenesis-related (PR) proteins. On the other hand, the overexpression was non-existent or reduced with the dual inoculation treatment. The positive influence of the pre-inoculation with P5 was also observed in the rest of analyses, e.g. the oxidative stress and concentration of total phenols and flavonoids increased in response to the pathogen inoculation, while no difference compared to control plants was observed in P5-*Ophiostoma* inoculated plants. As a conclusion, the defense responses induced by *Rhodotorula*-P5 could promote priming in *U. minor* plants, contributing to the enhancement of plant tolerance against DED.

## SESSION 13

### **49. CRISPR-Cas9 strategies for editing candidate genes in the Dutch elm disease pathogen *Ophiostoma novo-ulmi***

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The fungal tree pathogen *Ophiostoma novo-ulmi* is responsible of the Dutch elm disease. Despite the importance of this exotic invasive pest, we know little about the fitness and virulence factors of this pathogen. The BioSAFE project is growing the genomic resources to improve the understanding of the *O. novo-ulmi* biology and epidemiology. As part of this initiative, and in order to ascertain the function of identified candidate genes, we report on the development of clustered regularly interspaced short palindromic repeat (CRISPR)–CRISPR-associated protein 9 (CRISPR-Cas9)-mediated strategies to create target genes inactivated mutants in *O. novo-ulmi*. A key finding of this research is that transformation with a linearized plasmid expressing Cas9 and a selectable marker, along with synthetic guide RNA (gRNA), resulted targeted insertional gene mutation. 100% of the analyzed mutants integrated the linearized plasmid at the expected Cas9 double strand break of the target gene, with minor recombination events on flanks of the inserted plasmid. We refined the CRISPR-Cas9 by validating a plasmid-free CRISPR-Cas9-based gene editing method. We used commercially available purified Cas9 pre-complexed to guide RNA to form ribonucleoprotein (RNP) complex, that in combination with donor DNA (dDNA), generated in-locus insertions. RPN complex yielded more target mutants than plasmid-based CRISP-Cas9. Using qPCR, we showed that the number of dDNA integrated is directly proportional to the number of copies of the Cas9 expressing plasmid inserted into the genome. Most of the mutants are showing multiple insertions of the dDNA, with one displaying more than 80 copies of the dDNA. RPN complex-based CRISP-Cas9 led to significantly less integration of dDNA when compared to plasmid-based expression of Cas9. In conclusion, we demonstrated that *O. novo-ulmi* is amenable to CRISPR-Cas9 gene editing, which alleviates the previous lack of efficient directed-homologous recombination in this species. The incoming flow of genomic data coupled with the availability of a method for high throughput gene characterization will speed up our understanding of *O. novo-ulmi* pathogenicity.

## 50. Susceptibility of different ash species to Emerald Ash Borer (*Agrilus planipennis*) in the ash collection of the Main Botanical Garden in Moscow

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Collection of ash (*Fraxinus*) species at the Main Botanical Garden of the Russian Academy of Sciences in Moscow was destroyed by aggressive East-Asian invader – emerald ash borer *Agrilus planipennis* Fairmaire (EAB). Ash trees of the collection were planted in 1950-80s. Their most intensive recent dieback took place during the period of 2010-2014 when approximately 51% of trees died.

By methods of dendrochronological cross dating we found that in the Garden the first tree of *F. pennsylvanica* Marsh was killed by EAB in 1997. Peak of ash trees dieback in the northern and central regions of the city of Moscow took place at 2005-2007 and in its' southern regions - at 2008-2012. The last ash trees were killed in these regions at 2013 and at 2014 accordingly.

In 2014 during health evaluation of trees of 24 different ash taxons it was found that many of dead and weakened ash trees had EAB galleries and exit holes. EAB killed all or the majority of trees of *F. americana* L., *F. americana* var. *iodocarpa* Fern., *F. angustifolia* Vahl., *F. excelsior* L., *F. excelsior* "Aurea" (Pers.) Schelle, *F. excelsior* "Diversifolia" Lingelsh., *F. lanceolata* Borckh., *F. nigra* Marsh., *F. ornus* L., *F. oxycarpa* Willd., *F. pennsylvanica* Marsh. and *F. rotundifolia* Mill. Only few EAB galleries were registered on weakened trees of *F. americana* var. *joglandifolia* (Lam.) D.J. Browne and *F. mandshurica* Rupr. *F. biltmoteana* Beadle., *F. bungeana* DC., *F. chinensis* Roxb. (= *F. rhynchophylla* Hance), *F. excelsior* "Argenteo-variegata", *F. excelsior* "Nana", *F. latifolia* Benth. (= *F. oregona* Nutt.), *F. quadrangulata* Michx., *F. profunda* (Bush) Bush (= *F. tomentosa* Michx. L.), *F. sogdiana* Bge. and *F. velutina* var. *glabra* Rehd. appeared to be free from EAB attack.

During last 4 years EAB nearly disappeared from the city and from Moscow suburbs although many damaged ashes are still alive and are regenerating successfully.

**Acknowledgement.** The work was supported by the Russian Foundation for Fundamental Research (grant 17-04-01486).

## 51. Emerald Ash Borer (*Agrilus planipennis*) in European Russia: history of the invasion and current situation on the frontline

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Dendrochronological crossdating proved that emerald ash borer *Agrilus planipennis* Fairmair (EAB), East-Asian invader, appeared simultaneously both in North America (Michigan, USA) and in Eastern Europe (Moscow, Russia) in early 1990-s. The beetle itself was noticed because of its damaging activity much later: in 2002 (USA) and in 2003 (Russia). Since that time EAB has spread in European part of Russia over 12 administrative districts («subjects of the Russian Federation»). For a moment its secondary range there occupies approximately 250 thousand km<sup>2</sup> (~ ½ of territory of France) and spreads from the city of Yaroslavl at the North (57°37' N) to the Southern border of Voronezh District at the South (50°12') and from the city of Talovaya, Voronezh District (40°43' E) at the East to the city of Smolensk (32°02') at the West. The most Western locations of EAB are at 70 km from the border with Belorussia and only in 25 km from the border with Ukraine, so there is a large possibility that EAB already crossed the Russian border and can be found in Lugansk District of Ukraine and in Vitebsk District of Belorussia.

During last 7 years our team had a unique possibility to study EAB populations both in an epicenter of its secondary range in Europe (Moscow District) and in moving frontlines of invasion in Western and Southern directions. In these regions we received data on factors of population dynamics of EAB:

- climatic constrains;
- relations with different ash species;
- longevity of EAB generations;
- speed of EAB distribution;
- EAB/ash dieback (*Hymenoscythus fraxineus*) interactions on the newly discovered territory of their overlapping ranges;
- relations with predators and parasitoids;
- etc.

Although at frontlines of invasion the rate of parasitizing is very low, during last 5 years EAB nearly disappeared from Moscow District. Existing data demonstrated that EAB outbreak collapse was caused in major extent by local polytrophic parasitoid from genus *Spathius* Nees (Hymenoptera: Braconidae) who have switched to the new abundant host. This is a unique example when local biota only during a quarter of a century assimilated populations of the aggressive invader. It generates some optimistic expectations about the future of ash species in Europe



European part of Russia with invasive pest and pathogen (EAB and ash dieback) is a promising polygon for international cooperative effort of saving the world's ash forests.

**Acknowledgement.** The work was supported by the Russian Foundation for Fundamental Research (grant 17-04-01486).

## 52. Testing ash for resistance to dieback: an amateurish approach

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The work has been done within the framework of LIFE+ Nature project "Saving wooded Natura 2000 habitats from invasive alien fungi on the Island of Gotland, Sweden" (ELMIAS, NAT/SE/001139). The project is co-ordinated by Swedish Forestry Agency with Swedish University of Agricultural Sciences as one of the partners, responsible (among several others) for the Action 5: "Identification of dieback-resistant *F. excelsior* genotypes to create a database of at least 100 presumably dieback-resistant *F. excelsior* genotypes native to Gotland". Aims: i) search for visually healthy trees in severely damaged ash stands focusing on Natura 2000 sites; ii) select, measure, photograph and GPS record; iii) collect seeds from those and sow them on former agricultural land; iv) search for at least 1000 visually healthy 2-3 years-old saplings of ash in neighbouring disease-affected stands; v) dig out and out-plant those in the designated area; vi) continuously monitor health status of selected, sawn and planted ash trees. Preliminary results of the conducted work will be presented.

## **ABSTRACTS FOR POSTER PRESENTATIONS IN UPPSALA**

### **53. Pathogenicity and development of *Heterobasidion annosum* and *Heterobasidion parviporum* in functional sapwood of middle-aged coniferous and broadleaved trees**

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Artificial inoculation experiments have been used for more than 20 years to detect susceptibility of broad-leaved and coniferous tree species and still is used to test susceptibility to *Heterobasidion*. The aim of the research was to evaluate pathogenicity and development of *Heterobasidion annosum* and *Heterobasidion parviporum* in *Alnus incana* (L.) Moench, *Alnus glutinosa* L., *Betula pendula* Roth, *Populus tremula* L., *Fraxinus excelsior* L., *Larix decidua* Mill., *Picea abies* (L.) H.Karst, *Pinus sylvestris* L., *Quercus robur* L. Heterokaryotic isolates of *Heterobasidion annosum* (Fr.) Bref. (RV358) and *Heterobasidion parviporum* Niemelä & Korhonen (Rb 175) were used. Inoculations were made in May 2007. A total of 20 of each tree species were inoculated with *H. annosum*; whereas 20 of each tree species were inoculated with *H. parviporum*. For controls 10 of each tree species were used. The trees were inspected every year, but sampled twice: two and ten years after inoculation. At the first sampling trees were sampled using increment borer 3-5 cm above the inoculation point for evaluation of *Heterobasidion* in inoculated trees. At the second sampling trees were felled and after that dissected and following parameters were recorded: age of tree, stump diameter, tree DBH, tree height, decay diameter at stump level and length of decay column. Discs were examined for the presence of *Heterobasidion* conidiophores.

Only coniferous trees became infected. As somatic compability tests showed uncertain results, it raises the question about use of relatively old isolates in artificial inoculation experiments.

#### 54. Evaluation of the occurrence of biological control agent Rotstop and native strains of *P. gigantea* in commercially treated stumps one to three years after treatment

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Rotstop<sup>®</sup>, the biological control agent, composed of a suspension of spores of *Phlebiopsis gigantea* (Fr.) Jül. is used in large scale in the Europe for protecting conifer stumps from aerial infection by *Heterobasidion* species. In earlier studies, the occurrence of Rotstop isolate has been compared in treated and control stumps. In our research we compared the area occupied by the Rotstop strain with area occupied by native *P. gigantea* strains in treated conifer stumps, also formation of *P. gigantea* fruit bodies was evaluated.

The efficacy of Rotstop application on Norway spruce (*Picea abies* (L.) H.Karst) and Scots pine (*Pinus sylvestris* L.) stumps was determined in 3 stands one, two and three years after stump treatment. Stumps were treated in commercial thinning and the first observation was done one year later (2013) in October, the following observations were made in the autumn of 2014 and 2015. The area occupied by Rotstop strain, native *P. gigantea* and *Resinicium bicolor* (Alb. & Schwein.) Parmasto was analysed in total in 288 spruce and 296 pine stumps. Additionally, the incidence of *P. gigantea* fruit bodies was surveyed in 150 pine and 150 spruce stumps for three year period. Isolations from 157 discs were made to prove occurrence of Rotstop. Development of *Heterobasidion* spp. was marked.

One year after stump treatment incidence of *P. gigantea* were detected in 91 % of pine and 45 % of spruce stumps. The area covered by fungus varied from 0.2 to 99.0 % (on average  $48.2 \pm 36.6$  %). *Heterobasidion* spp. occurred in 14 % of spruce stumps. Occurrence of *P. gigantea* decreased at the second year (38 % of pine and 33% of spruce stumps were colonized; area varied from 0.1 to 72.0 %, on average  $10.7 \pm 16.3$  %). *Heterobasidion* spp. occurred in 12 % of analysed spruce stumps. Three years after treatment only 16 % of pine stumps and 31 % of spruce stumps were colonized by *P. gigantea*. Frequency of *Heterobasidion* spp. was similar as in previous years in spruce and pine stumps 15 % and 1 %, respectively. In total, 66 % of discs were colonized by *P. gigantea*, 85 % of them represented Rotstop isolates and 15 % native strains of *P. gigantea*. Fruit bodies were most abundant on pine and spruce stumps two years after treatment, 32 % and 5 % respectively.

## 55. Effect of silver and copper nanoparticles on the control of damping-off disease and growth in Scots pine (*Pinus sylvestris* L.) in the nursery of Sychowo Forest District, Poland

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Nanoparticles are gaining ever-wider application in plant production (for both agriculture and forestry), in the role of pesticides, as well as stimulators of plant growth and resistance. In line with that, the work described here sought to determine the efficacy of action of nanoparticles of silver and copper (AgNPs and CuNPs), used as seed dressings or made subject to foliar applications, in affording protection from parasitic damping-off disease among soil-grown seedlings of Scots pine in a forest nursery. Experiments also assessed the influence of nanoparticles on the growth of the plants potentially safeguarded in this way. Nanoparticles were used in seed soaking and foliar spraying at concentrations of 50 ppm. Pines treated with fungicides (Zaprawa Nasienna T 75 DS, Acrobat MZ 69 WG, Topsin M 500 SC, Gwarant 500 SC, Thiram Granuflo 80 WG o Signum 33 WG) or not protected at all were used in comparison. In each treatment (AgNPs, CuNPs, fungicides or not protected), seedlings were inventoried 6 weeks after seed sowing and at the end of the growing season, while all individuals on 80 (1-metre long) segments of seed row were counted. At the end of the growing season, seedling growth parameters like shoot length, root-collar diameter, root length and dry mass of shoots and roots were determined. In the event, seedlings treated with nanoparticles were found to have longer root systems, of greater dry mass; but also only more weakly-developed above-ground parts (both heights and dry masses being limited); in comparison with young Scots pines that had been fungicide-treated or were in the control (i.e. unprotected) variant. The consequence was a significantly more favourable ratio of mass of stems to mass of roots where nanoparticle treatment had been applied. The effectiveness of the protection extended to the germination and first-growth stages up to 6 weeks from the time of sowing was furthermore shown to be greatest where AgNPs had been applied, while at season's end there were comparable results among pines treated with either nanoparticles or fungicides. Our results thus indicate that nanoparticles limit damping-off disease in pine seedlings (AgNPs more effectively than CuNPs), with that effectiveness also proving comparable with that noted for the fungicides applied traditionally.

**Keywords:** seedling growth, disease control, *Pinus sylvestris*, damping-off disease, nanoparticles

## 56. The influence of stress factors on the susceptibility of different tree species to *Heterobasidion annosum*

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*Heterobasidion* spp. cause one of the most important tree diseases in the boreal hemisphere. In Poland *H. annosum* occurs over the whole country and infests the widest range of tree-hosts, causing root rot. Extreme climatic and stress conditions, which recently occur increasingly often, could decrease plant resistance and increase pathogen activity. The aim of the study was to determine the influence of selected stress factors on the susceptibility of trees to *H. annosum* infection.

### Materials and methods

Six tree species were used in the experiment: Scots pine, Norway spruce, European fir, larch, sessile oak and birch. Seedlings, taken from a forest nursery, were planted in 7.5-l pots. After one year of acclimatization, seedlings were subjected or not subjected to stress factors: root pruning (30% reduction), defoliation (2/3 reduction), nutrient deficiency (growth in river sand), nitrogen excess (three times more than usual). After one month, seedlings were inoculated with woody sticks colonized by *H. annosum*. Control seedlings were inoculated or not inoculated with sterile sticks. In the experiment, 50 seedlings of each tree species and 10 isolates of *H. annosum* were used in all combinations (stress factor x tree species x *H. annosum* isolate). After three months of growth, seedlings were removed and washed, and necrosis in the sapwood was measured above and below the inoculation point. The length of necrosis was used as an indicator of wood colonization by *H. annosum*. The pathogen was re-isolated from the necrotic tissue. Results were analysed with one-way and multifactorial ANOVA ( $P < 0.0001$ ), and HSD Tukey and NIR post-hoc tests ( $P < 0.05$ ).

### Results

Necrosis developed in all stems inoculated with *H. annosum*. Re-isolation proved the presence of *H. annosum* in necrotic tissue. Tree species subjected and not subjected to stress factors responded differently. *Heterobasidion annosum* caused most extensive necrosis in trees subjected to stress factors (16.22 mm), significantly different from that in trees not subjected to stress factors (13.80 mm). The most extensive necrosis was in European fir (15.15 mm), Norway spruce (11.64 mm) and Scots pine (10.81 mm). There was, however, extensive necrosis also in European fir not subjected to stress factors (16.9 mm). Scots pine subjected to nutrient deficiency and Norway spruce subjected to defoliation were the most susceptible to *H. annosum* infection. Susceptibility of European fir to *H. annosum* infection and its development seemed to increase with nutrient deficiency and nitrogen excess. Stress factors moderately affected the susceptibility of sessile oak and did not affect

the susceptibility of birch and larch. Nutrient deficiency was the most important factor that increased the susceptibility to *H. annosum* infection.

**Conclusions:**

- Effects of stress factors on the susceptibility of trees to *H. annosum* infection depends on the type of factor.
- Tree species known to be resistant to *H. annosum* (e.g. sessile oak) may become susceptible in stress conditions.
- Nutrient deficiency and defoliation can contribute to spread of *H. annosum* in Scots pine, Norway spruce, European fir or even sessile oak stands.
- Nitrogen excess and reduction of roots may not contribute to spread of *H. annosum*.

## 57. The tree-fungus beetles collected onto the sawdust substrate with the mycelium of selected fungi species in the forests of the Experimental Forest Station in Rogów (Central Poland)

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The study presents data on tree-fungus beetles caught in traps, in which the bait substance was sawdust substrate overgrown by the mycelium of 7 species of lignicolous fungi (*Bondarzewia mesenterica*, *Fistulina hepatica*, *Fomitopsis rosea*, *Grifola frondosa*, *Hericium coralloides*, *H. flagellum*, and *Meripilus giganteus*). The research was carried out in central Poland, in the oak-hornbeam forest *Tilio-Carpinetum stachyetosum*, in the Forest Experimental Station in Rogów. One research season yielded the catch of 133 beetle species ecologically connected with lignicolous fungi, including 60 mycetobiontic species. The most numerous species were: *Trixagus carinifrons*, *Corticicara gibbosa*, *Dinerella ruficollis*, *Rhizophagus bipustulatus*, and *Aulonothroscus brevicollis*. Two autonomous assemblages of tree-fungus beetles were isolated in the collected material. The first one included beetles associated with brown rot of wood, and the second one – those associated with white rot.

**Keywords:** Coleoptera, mycetophilic beetles, mycetobiontic beetles, wood-decay fungi, brown rot, white rot, Poland



## 58. *Diplodia* tip blight in Germany: ecological and molecular characteristics of the tree pathosystem

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The fungus *Sphaeropsis sapinea* (syn. *Diplodia pinea*) causes tip dieback, stem cankers and blue stain mainly on pines but also on other conifers worldwide. In Germany, mainly *Pinus sylvestris* and *P. nigra* are affected by the ascomycete, which may kill shoots, branches and the entire tree. The disease has been able to spread easily worldwide because it has a symptomless endophytic life stage in its disease cycle. Symptoms come visible when trees have been weakened by stress, such as drought or insect bites. The presence of the disease has been observed more frequently over the past years in pine forests in Germany. In future, changes in climatic conditions will increase this fungus to become actively pathogenic in conifer forests, thus actions to restrict the spread of *Sphaeropsis sapinea* species must be taken immediately to secure the health of pine-dominated forests. However, there is currently no clear method for stopping its spread. This project aims to study what is the level needed for this fungi to switch from an endophytic to the pathogenic lifestyle. We will combine several different aspects: diversity studies, transcriptomics, inoculation experiments, and regular inventories in the field. Our first aim is to compare the fungal composition of healthy pines with those of diseased trees by Next Generation Sequencing and traditional culturing methods. With this poster, we present the current disease stage of *Diplodia* tip blight in Germany, the research co-operation with Northwest German Forest Research Institute and our future plans for this study.

## 59. Wood decay of coniferous species caused by the mycelium of *Neolentinus adhaerens* (Alb. & Schwein.) Redhead & Ginns under laboratory conditions

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*Neolentinus adhaerens* (Alb. & Schwein.) Redhead & Ginns is a saprotrophic species which belongs to the family Polyporaceae, order Polyporales (Basidiomycota). This fungus causes brown-rot decay of the wood of coniferous trees. Pure culture of *N. adhaerens* mycelium was obtained from a fragment of the fruit body growing on a lying fir log. The aim of the study was to investigate, through laboratory decay tests, the ability of *N. adhaerens* to degrade wood of *Abies alba*, *Picea abies*, and *Pinus sylvestris*. After sterilization, wood samples were put into Kolle flasks with the mycelium of *N. adhaerens* on MEA medium. Subsequently, the flasks were incubated for 120 days, in the constant temperature of 22°C. After the incubation period, the wood samples were dried in 105 °C until they reached a constant weight. The following average percentage wood weight losses resulting from decay caused by the mycelium of *N. adhaerens* were identified: *A. alba* (15.05%), *P. abies* (15.42%) and *P. sylvestris* (8.10%). The average percentage weight loss of pine wood differed from average percentage weight losses of fir wood and spruce wood. Additionally, the distribution of *Neolentinus adhaerens* in Poland has been presented.

**Keywords:** Polyporaceae, *Neolentinus*, *Abies*, *Picea*, *Pinus*, wood decomposition, brown rot

## 60. Distribution of *Heterobasidion* and *Armillaria* root rot fungi in Vallombrosa fir forest, Italy

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On 5th March 2015, the Natural Reserve of Vallombrosa (Tuscany, Italy) was hit by a severe windstorm that caused considerable damages to the forest stands. The chronic root rot disease caused by *Heterobasidion annosum* s.l. and *Armillaria* spp. is a key factor affecting the vitality of silver fir plantations in the region. In order to delineate the spatial distribution of both species complexes, a systematic sampling was undertaken at the intersection of 52 grid points. Identification of different fungal species from soil and fungal samples was accomplished by morphological traits and/or DNA-based methods (TSCP, nested PCR, RFLP analysis). The widespread presence of *H. abietinum* was confirmed, concentrated in conifer stands. The high diversity of *Armillaria* spp. was detected in a wide range of habitats. Unfavourable environmental conditions in the area predispose *Abies alba* trees to fungal infection and might decisively influence the disease outcome. A partial objective of our study was to assess the general health condition of the Vallombrosa forest, based on a survey of damages on trees. The obtained results might help forest managers to control the pathogens.

## 61. Diversity of invertebrate in *Heterobasidion* spp. fruit bodies on decayed spruce wood

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Saproxyllic basidiomycetes does not only provide microhabitats to many invertebrate species but also cause great silvicultural losses. Root rot mainly caused by a pathogenic fungus *Heterobasidion* spp. currently is one of the greatest challenges. Information about invertebrates inhabiting root rot fruit bodies is sketchy. It is important to determine if by controlling root rot, invertebrates inhabiting it are not affected, thereby reducing favorable environmental conditions for them. The goal of this study was to determine invertebrate diversity in fruit bodies of *Heterobasidion* spp. on decayed spruce wood, and factors influencing it. Data was collected using Tullgren funnel traps in the autumn of 2016. In total 7341 invertebrate individuals representing more than 16 orders were collected from 134 *Heterobasidion* spp. fruit bodies, spruce wood without root rot and soil samples. Samples were dominated by mites, springtails, beetles, millipedes and true flies. None of the invertebrate species found inside *Heterobasidion* spp. fruit bodies are red listed. Studied substrate differed by the number of invertebrate individuals and structure. Although invertebrate diversity was high in fruit bodies, their greater diversity was found in wood samples. We have found that invertebrate diversity in fruiting bodies of root rot fungus is connected to a log diameter on which fruiting body is growing – species diversity was higher on a bigger diameter logs. Wood and fruiting body decay stage and weight of sample had a positive effect on invertebrate diversity. For the first time, comprehensive data is available on *Heterobasidion* spp. fruit bodies inhabiting invertebrates.

## 62. The effect of silver and copper nanoparticles on the growth and mycorrhizal colonisation of Scots pine (*Pinus sylvestris* L.) in a container nursery experiment

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As certain research points to a possibility of nanoparticles being used as fertilisers, growth stimulators, promoters of plant resistance or pesticides, the work detailed here sought to determine the influence of such nanoparticles of silver and copper (AgNPs and CuNPs) on growth parameters, and on spontaneous mycorrhizal colonisation of roots, in 2-year-old container-grown seedlings of Scots pine. The nanoparticles were the subject of foliar applications made through two growing seasons, four times a season, at concentrations of 0, 5, 25 and 50 ppm. These different treatments were the subject of TEM-based comparisons of ultrastructure characterising the needles, stems and roots of the treated or untreated pines. The CuNPs deployed were found to stimulate mycorrhizal colonisation at all concentrations, though growth of the seedlings was only promoted where the concentration was 25 ppm. Higher (25 and 50 ppm) concentrations of AgNPs inhibited the formation of mycorrhizae, although the lowest (5 ppm) concentration did give rise to an increase in both mycorrhizal colonisation and the dry mass of roots. Ectomycorrhizal fungi reported were of the species *Thelephora terrestris*, *Suillus bovinus* and *Sphaerospora brunnea*. TEM results comparing with control (untreated) needles revealed changes in chloroplasts from lens-shaped to spherical, with an increase in the number of plastoglobules and the presence of large osmophilic globules in cytoplasm solely associated with needles of the pines receiving a 50 ppm concentration of nanoparticles. In contrast, it did not prove possible to note ultrastructural changes in stems and roots associable with the applications of NPs. Overall, results point to the possible use of CuNPs and AgNPs as stimulators of growth in general and mycorrhizal colonisation in particular, among container-grown Scots pines. However, it is clear that further work is needed if optimal doses and concentrations are to be determined.

**Keywords:** nanoparticles, ectomycorrhizae, toxicity, growth stimulation

### 63. Situation of *Armillaria* spp. and *Heterobasidion* spp. in Slovenia

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Species of the genus *Armillaria* and *Heterobasidion* are among the most common causes of stem and root rot in Slovenia. *Armillaria* spp. infects deciduous and coniferous trees, while *Heterobasidion* spp. mainly threatens spruce, pines, and fir. On the basis of the data about the sanitary felling of infected trees, we estimated the current state and calculated the proportions represented in total felling, total sanitary felling, total sanitary felling due to diseases, in wood stock, and in increments from 2013 to 2017. Since 2014, there has been a constant increase in the sanitary felling of deciduous and coniferous trees due to infections with *Armillaria* spp. In 2017, 32,849 m<sup>3</sup> of timber were harvested due to *Armillaria* spp. Given the present situation, we assume that the amount of sanitary felling will continue to increase, but it will not account for large shares in wood stock or increment (<1%). In 2017, sanitary felling of infected conifers represented 27.6% of all sanitary felling due to diseases. In the case of deciduous trees, the share was lower, i.e. 7.1%. *Armillaria* spp. was the main cause of sanitary felling due to disease (51.9%) in the Postojna forest management unit (FMU), while elsewhere shares of up to 10% were recorded. With *Heterobasidion* spp., the amount of felling is decreasing over the years. In 2017, 33,922 m<sup>3</sup> of wood, accounting for 15.7% of the total sanitary felling due to disease, were felled due to *Heterobasidion* spp. A comparison of the felling of Norway spruce, silver fir, and Scots pine due to *Heterobasidion* spp. shows the different proportions of felling within the total sanitary felling due to the diseases. In spruce, it is on average 79.5%, in fir 12.9%, and in Scots pine 34.3%. We assume that the volume of timber harvested due to *Heterobasidion* spp. will gradually decrease over the years due to the lower wood stocks of spruce, which has recently been hit by numerous natural disasters and infestation of bark beetles.

**Keywords:** sanitary felling; *Armillaria* spp.; *Heterobasidion* spp.; root rot; root disease; Slovenia

## 64. Effect of injuries on stability of Norway spruce

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Populations of large game as moose and red deer are continuously increasing in Europe. Consequently, also the frequency of bark-stripping wounds in pole-stage Norway spruce is rising, as demonstrated by National forest inventory data. Previous studies have shown, that decay occurs in 13-50% of investigated wounded (exposed sapwood 7 – 6142 cm<sup>2</sup>.) *P. abies* trees depending on study site (mean 26.7%). The most commonly isolated fungi were ascomycetes *Neonectria fuckeliana*, *Sarea difformis* and *Phialocephala* sp., and basidiomycetes *Cylindrobasidium evolvens* and *Amylostereum areolatum* (Burneviča et al., 2016; Arhipova et al., 2015), thus potentially reducing the wind resistance of the trees. To estimate this impact, we applied static pulling tests and measured pulling force and inclination at the tree base and the height of 5m. To avoid any wind effect on the measurements, trees were topped prior the winching. No significant differences in morphometric parameters of above-ground parts were observed between browsed and un-browsed trees. However, results revealed statistically significant ( $p < 0.05$ ) reduction in all calculated parameters for bark-stripped trees compared with unaffected ones. It indicates a lasting (injuries occurred 17 years ago) negative effect of bark stripping on individual tree stability.

**Keywords:** tree pulling, Norway spruce, browsing damage, wind damage

**Acknowledgement.** Study was carried out in JSC Latvija valsts meži project “Forest management risks: prognosis and minimization”.

## 65. Practical solutions for the surveillance and the containment of the invasive forest pathogen *Heterobasidion irregulare* in Italy

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The North American root rot agent of pines *Heterobasidion irregulare* Garbel. & Orosina was accidentally introduced in central Italy during World War II. The pathogen has become invasive and is currently distributed in pine and oak stands along 105 km of coast around Rome, often in association with significant mortality of Italian stone pine (*Pinus pinea* L.). Based on its observed and potential impact, *H. irregulare* has been included by the European and Mediterranean Plant Protection Organisation (EPPO) in the A2 list of pests recommended for regulation. In the frame of the EU Project EMPHASIS (grant agreement 634179), a comprehensive research is being providing practical solutions for the monitoring and the containment of the invasive pathogen, including local eradication. Usually, the most cost-effective options to control *Heterobasidion* spp. rely on the protection of freshly cut stump surfaces with biological or chemical treatments and on timing logging operations in periods of the year characterized by a low risk of stump infection. Stump removal may also be effective, although this operation may be costly and time consuming, and may be unfeasible due to technical constraints. Here we report on: i) the development and optimization of an efficient molecular diagnostic tool based on Loop-mediated isothermal AMPLification (LAMP) for the detection of *H. irregulare*, ii) the screening of local biological control agents [e.g. Italian isolates of *Phlebiopsis gigantea* (Fr.) Jülich and *Peniophora* sp.] effective against the pathogen, and iii) the feasibility and effectiveness of two eradication solutions, i.e. felling of infected trees combined with stump grinding, and uprooting of infected trees by using an excavator. Additionally, we assessed the colonization ability of *H. irregulare* up into the stem of infected trees, to determine whether the movement of wood from the stem may represent a phytosanitary risk. Results of this project combined with all data previously achieved on the invasion of *H. irregulare* are contributing to the preparation of two EPPO Standards on diagnostics (PM7) and on national regulatory control systems (PM9) aimed at providing guidelines for the surveillance and containment of *H. irregulare* in Europe.

**Acknowledgement.** This work was funded by the European Commission in the frame of the EU Project EMPHASIS (grant agreement 634179)



## 66. Survey and relative abundance of *Heterobasidion* species distribution in coastal British Columbia's forests

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The basidiomycete fungus *Heterobasidion annosum sensu lato* is the most serious pathogen of conifer forests in the northern hemisphere. The fungus infects its hosts following deposition of airborne spores through fresh cut stumps or wounds and spreads through the root system by root grafts or root contact to adjacent trees, causing root and butt rot, which result in expanding root disease centers. The species complex comprises of two North American (*H. occidentale* and *H. irregulare*) and three Eurasian (*H. annosum sensu stricto*, *H. parviporum* and *H. abietinum*) species. The North American *Heterobasidion* spp. are geographically separated in Canada, with *H. irregulare* primarily infecting pine trees in Ontario and Quebec, and *H. occidentale* infecting several conifers including western hemlock, Douglas-fir, amabilis fir, western red cedar, and Sitka spruce in coastal British Columbia (BC) forests. In order to determine the relative abundance of the two North American *Heterobasidion* species in coastal BC forests, a field survey was undertaken by trapping airborne spores on a semi-selective media in Petri plates that were exposed in selected areas. Spore traps were set in locations from which the Pacific Forestry Centre (PFC) Herbarium samples had previously been collected. To date, the survey included sites on Vancouver Island and Haida Gwaii. We utilized microscopic, cultural and molecular diagnostic tools to identify putative *Heterobasidion* species based on the presence of its conidial stage *Spiniger meineckellus* and PCR species-specific primers developed at PFC to identify and confirm the *Heterobasidion* at the species level. Initial results show that *Heterobasidion* is still present in areas where *Heterobasidion* PFC Herbarium samples were previously collected, providing an excellent and easy method to survey the presence of *Heterobasidion* without the presence of fruiting bodies. A collection of close to 600 *S. meineckellus*-forming colonies have been isolated and maintained for long-term storage for further research. In addition, using species-specific PCR primers, we were able to document for the first time the occurrence of *H. irregulare* isolated from basidiocarps collected from ponderosa pine and eastern white pine in Okanagan, Naramata and Summerland, southern interior of BC, respectively.

**Keywords:** *Heterobasidion annosum*, species complex, root and butt rot, *Spiniger meineckellus*, spore dispersal, PCR detection, species typing

## 67. Monitoring of *Phlebiopsis gigantea* on commercially treated spruce and pine stumps in Latvia

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*Heterobasidion* spp. is one of the most important pathogen causing root and butt rot in conifers worldwide. Several control methods including biological preparation containing spores of *Phlebiopsis gigantea* are used to limit spread of *Heterobasidion* spp. Biological control agent Rotstop® is commercially used in pre-commercial thinning to limit spread of *Heterobasidion* spp. in Latvia since 2008. The aim of the study was to assess the quality of conifer stump treatment with Rotstop in Latvian State forests. Monitoring of stump treatment was started in 2008 but more detailed data have been obtained since 2013.

In total, 790 pine (*Pinus sylvestris*) and 424 spruce (*Picea abies*) stumps have been evaluated since 2013. Wood discs were collected to evaluate the area occupied by *P. gigantea*. The fungus was isolated from wood discs to confirm the treatment of Rotstop and assess frequency of local *P. gigantea* strains in conifer stumps. On average, 94.7 % (87.6 - 100 %) pine and 61.8 % (42.1 - 81.6 %) spruce stumps were colonized by *P. gigantea*. Area occupied by *P. gigantea* varied between tree species. On average, 57.9 % (0.05 - 99.8 %) of pine stump surface area was occupied by *P. gigantea*, whereas on spruce stump surface area only 11.2 % (0.03 - 96.8 %) was colonized. In 17.7 % pine and 11.5 % spruce stumps both, *P. gigantea* used in Rotstop and local *P. gigantea* strains were obtained. Studies in Latvia are focused on finding local *P. gigantea* strains that could be used in spruce stands and would be more effective than *P. gigantea* used in Rotstop.

## 68. Development of *Phlebiopsis gigantea* fruit bodies on large diameter log pieces of Norway spruce and Scots pine

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*Phlebiopsis gigantea* is a common wood-decay fungus in conifer forests of Northern hemisphere. It is well known for its use as a biological control agent against the infection of fresh conifer stumps by spores of root rot fungus *Heterobasidion*. Presence of sporulating fruit bodies of *P. gigantea* in forest favours natural protection of stumps against *Heterobasidion*. The aim of this research was to assess the role of large diameter log pieces of Norway spruce and Scots pine in fruiting of *P. gigantea*.

The experiment was established in the beginning of July 2013 in eastern part of Latvia. On five sample plots, 60 healthy spruce trees and 60 trees containing *Heterobasidion* root rot were cut. Also 28 healthy pine trees were cut. The stems were cut to four 70 cm long pieces, starting from root collar, and left in forest. In total 160 log pieces of Norway spruce and 48 log pieces of Scots pine were used in the experiment. Cutting surfaces of half of the logs were manually treated with spore suspension of *P. gigantea* of Latvian origin. Further, in each category the bark of half of logs was damaged, imitating the effect of silvicultural practices in forest. Bark of the other half was left intact. Each log piece was numbered and marked with plastic band. The log pieces were surveyed for the presence of *P. gigantea* fruit bodies in autumn of the following years (2014, 2015 and 2016).

In 2014, *P. gigantea* fruit bodies were present on 88% of pine log pieces and on 53% of spruce log pieces. The difference was statistically significant. Corresponding figures in 2015 were 54% and 48%, and in 2016 6% and 21%. Area occupied by *P. gigantea* fruit bodies varied greatly between the logs – from less than 1% to 80%. The fruit bodies were more abundant on cutting surfaces than on lateral surfaces of the log pieces. The occurrence of fruit bodies on logs with bark damages did not differ significantly from logs with intact bark. On pine logs, treatment with *P. gigantea* had no significant effect on the occurrence of fruit bodies. On spruce logs, after one and two years, *P. gigantea* fruit bodies were more present on treated logs, but after three years there were no significant differences between treated and untreated logs. *P. gigantea* fruit bodies were less present on spruce logs containing *Heterobasidion* butt rot, compared to healthy logs. The occurrence of *P. gigantea* fruit bodies decreased with time.

## 69. Management of *Armillaria* using combinations of biological controls and plant defence activators

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*Armillaria* is a globally distributed and economically important root pathogen, primarily of woody species. Urban & amenity trees/shrubs are one group under particular threat with the functional benefits (pollution and flood reduction, shade, cooling) of protecting these trees for humans and urban environments increasingly recognized. Consequently, mature trees in populated areas are commonly recognized to have significant economic value. Controls economic in forestry i.e. removal, do not provide a significant level of protection for at risk trees in urban and amenity situations. Preventative chemical controls have proven unreliable and face environmental concern. Despite a relatively rich history of study, no single biological control organism for *Armillaria* has emerged. A combination of various biological control organisms (*Trichoderma*, *Bacillus*, *Pseudomonas*), and products that stimulate plant immune systems (chitin, phosphite, biochar), has never been studied and may provide improved control. Each system can provide direct and plant mediated impacts on *Armillaria*, with potential for additive or synergistic interactions. Common biological controls and plant immune stimulants have been variously tested in *in vitro* antagonism experiments, pot trials, and a naturally infected woodland site with promising results.

## 70. Identification of candidate genes associated with the resistance QTLs in Norway spruce through a combined analysis of transcriptomics and genetic maps for *Pinaceae*

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The *Heterobasidion annosum* species complex is the most damaging forest pathogen on Norway spruce (*Picea abies*), posing a major threat to Swedish forest productivity. Thirteen Quantitative Trait Loci (QTLs) related to *Heterobasidion*-resistance, have been identified in Norway spruce (1), and one of these QTLs has been validated (2). However, to allow for efficient resistance breeding more markers are needed. Thus, the goal of our study is to identify further candidate genes associated with the resistance-QTLs. To achieve this we identified 121 gene models associated with the QTLs using a high-density gene-based composite map (3). We further determined the transcriptional responses of these gene models at three and seven days after wounding, or inoculation with *Heterobasidion parviporum*, hypothesizing that genes which are likely to be important for controlling spread of *H. parviporum* are likely to be more strongly regulated by inoculation than by wounding alone. We found 84 gene models to be expressed and 54 of those to be differentially expressed between wounding and inoculation. Based on the RNA-analysis PaNAC04 and paralogs (4), appear to be strong candidates for further validation and functional genetic studies. The genes were found to be associated with one of the QTL regions and were highly induced in response to *H. parviporum* compared to wounding.

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## ABSTRACTS FOR POSTER PRESENTATIONS IN VISBY

### 71. Biocontrol potential of forest tree endophytes

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In the natural forest environment, the spread of pathogens may have dramatic effects on ecosystem functioning. To successfully control devastating forest pathogens, application of endophytes as biocontrol agents is an emerging area of research. There are several ways by which endophytic microorganisms can protect their tree hosts against pathogens. Endophytes promote plant growth by producing beneficial secondary metabolites (e.g. phytohormones) or providing nutrients (e.g. phosphorus). Endophytes can compete with pathogens and herbivores by successfully utilizing available substrates (colonization of shared niche can restrict pathogen invasion), or endophytes can produce antagonistic metabolites. Endophytes can enhance plant resistance by triggering and priming host defensive reactions. Endophytes could provide several opportunities for application in integrated pest management (IPM) to gain sustainable forestry practices. To utilize endophytes as biocontrol agents, the mechanisms behind the possible mode of action should be determined. Novel advances in cultivation-independent techniques including next generation sequencing technology (NGS), association analyses and network inference modelling will greatly facilitate identification of endophytes and unravel potential beneficial functions of endophytic communities. A further understanding of these mechanisms could help to minimize the use of environmental harming chemicals in plant and forest tree protection. Here we propose simple guidelines that could facilitate the use of root fungal endophytes as biocontrol agents and simultaneously study their ecological functions.

**Keywords:** endophytes, biocontrol, forestry

## 72. The microbial diversity and structure in peatland forest in Indonesia

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Tropical forest ecosystem plays very crucial part for controlling the universal carbon cycle and identification of microbial decomposers is vital for understanding organic matter transformation in these ecosystems. However, little is known about microbial structure and their essentiality in tropical peat land forest compared to other terrestrial habitats. In this study, we examined the bacterial and fungal community structure and diversity in well mixed swamp forest, disturbed peat forest and mineral forest in Central Kalimantan, Borneo Indonesia by using 454 pyrosequencing technology. The results showed that the natural peat soil had the highest fungal species richness (Chao1), which was significantly higher than that in the other two sites ( $P < 0.05$  and  $P < 0.05$ ), respectively. The fungal community structure in natural peat soil differed significantly from the mineral soil ( $P = 0.04$ ) and the disturbed peat soil ( $P = 0.039$ ), respectively. Ascomycota was the most abundant phylum (40.5%) followed by Basidiomycota (18.8%), Zygomycota ( $< 0.1\%$ ) and Glomeromycota ( $< 0.1\%$ ). Linear discriminant analysis coupled with effect size (LEfSe) showed that Ascomycota dominated in natural peat soil (LDAs=5.35,  $P < 0.05$ ). At genus level, *Gliocephalotrichum* was more abundant in natural peat swamp forest (LDAs=4.05,  $P < 0.05$ ), whereas *Penicillium* (LDAs=4.02,  $P < 0.05$ ) and *Gymnopilus* (LDAs=4.38,  $P < 0.05$ ) were more abundant in the mineral and the disturbed peat soil, respectively. Functionally, wood-degrading fungi were commonly represented across all the samples including the order *Polyporales*, especially in natural peat soil. Pathotrophic fungi *Metarhizium* and *Pestalotiopsis* had higher abundance in disturbed peat soil. Genera *Perenniporia* with whit-rot ability had higher abundance in disturbed peat soil, while the brown-rot *Fusarium* and *Coniophora* were only found in natural peat soil. The diversity of bacteria in disturbed peat soil significantly higher than the other two sites ( $P < 0.05$ , respectively). The bacterial community structure in natural peat soil significantly differed from the disturbed peat soil ( $P = 0.045$ ). Among the assigned 11 bacterial phyla, *Proteobacteria* (43.8%) were the most abundant phylum followed by Acidobacteria (32.6%). No significant differences were found for each phylum among sites. In total, 58 bacterial genera were obtained, of which, *Methylocystis*, *Telmatospirillum*, *Syntrophobacter* and *Sorangium* were more abundant in disturbed peat soil, while *Nevskia* and *Schlesneria* were more abundant in mineral soil and natural peat soil, respectively. In conclusion, the natural peat forest harbors more divers microbes and the land-use of peat land can change the microbial diversity and structure. This provides useful information on management use of the peat land forest in tropical area.

**Keywords:** tropical peatland, microbes, community structure, diversity, 454 pyrosequencing

### 73. Hormonal profiling of resistant and susceptible interactions between *Castanea* spp. and *Phytophthora cinnamomi*

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Mechanisms underlying resistance to *Phytophthora cinnamomi* in *Castanea sativa* are little understood. Recent studies related resistance of chestnuts to a higher constitutive and induced defense gene expression; however, hormonal signaling pathways upon infection are unknown. Within the project 'AGL2014-53822-C2', the constitutive and *P. cinnamomi*-induced hormonal profile of a resistant (commercial *C. sativa* x *C. crenata* 111-1 hybrid) and a susceptible (native *C. sativa* Cs14) clones were assessed. At 0 and 9 dpi, hormones, soluble sugars (SS), total polyphenols (TP) and condensed tannins (CT) were analyzed in leaves and roots (n=3). The resistant genotype showed higher constitutive content of ABA, JA, JA-Ile and TP in leaves, and SA in roots, than the susceptible genotype. Upon inoculation, SS increased in leaves and ABA, JA, JA-Ile and TP increased in roots (and decreased in leaves) of the resistant genotype. No change in hormone content was detected in leaves of the susceptible genotype, but a slight increase of SA and JA, and a decrease of TP in roots were observed. Resistance of plants to *P. cinnamomi* was not associated to the classical ABA-SA antagonism but to a particular leaf-root hormonal homeostasis pattern, in which several defense hormones accumulated in roots. Results indicate an efficient defense signaling in the resistant genotype which probably causes less efficient effector secretion by the pathogen. The exclusive and substantial increase of JA-Ile in roots of the resistant genotype could be related to the synthesis of antioxidant metabolites that may regulate oxidative stress by ROS scavenging. Results highlight the relevance of hormonal signaling pathways, in particular the role of JA-Ile and ABA, in explaining the mechanisms of resistance of *Castanea* to *P. cinnamomi*.



## 74. Resilience of *Castanea sativa* to stress factors related to global change

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The influence of changing scenarios of temperature on the performance and susceptibility of *Castanea sativa* to *Phytophthora cinnamomi* is ignored. This panel informs about four experiments to be carried out within the framework of a project and a PhD thesis. The following hypotheses will be tested: (i) 'prolonged and punctual episodes of high temperatures increase the susceptibility of *C. sativa* to *P. cinnamomi*'. For this, seedlings and commercial clones of chestnuts will be subjected to different temperatures, in accordance to several scenarios predicted by the IPCC, and further challenged with *P. cinnamomi*; (ii) 'there is a latitudinal gradient related to the differential response of *C. sativa* to high temperatures and *P. cinnamomi*'. Seedlings from several populations and countries will be characterized by EST-SSR markers related to temperature responses and submitted to the most unfavorable scenario tested under the first hypothesis; (iii) 'high temperatures and water stress probably interact when defining the susceptibility of *C. sativa* to *P. cinnamomi*'. Offspring of chestnut trees from populations of central Spain will be subjected to combining temperature x water stress treatments and challenged with *P. cinnamomi*; (iv) 'in the market there are genotypes tolerant to the combination of adverse factors of global change'. Commercial clones and eight genotypes previously tested for their tolerance to water stress and *P. cinnamomi* will be subjected to the worst scenario of the third hypothesis. This work aims to improve in the medium term the socioeconomic situation of areas in which *C. sativa* is grown by providing owners with plant material adapted to the current conditions of global change.

## 75. Xylem anatomy of offspring of two *Ulmus minor* trees tolerant to *Ophiostoma novo-ulmi*

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The vascular pathogen *Ophiostoma novo-ulmi*, causal agent of Dutch elm disease (DED), develops in xylem vessels and causes cavitation, hydraulic dysfunction, leaf wilting and tree death. Vessel size and other constitutive xylem anatomical parameters have frequently been related to elm susceptibility to DED. However, the way that the mother tree modulates the xylem anatomy in response to infection has not been quantified yet. Two tolerant *U. minor* trees were artificially crossed with the same susceptible *U. minor* clone, and their seedlings planted in an experimental plot at Puerta de Hierro Forest Breeding Center (Madrid, Spain). Seedlings were inoculated in 2014 and 2015 with an aggressive strain of *O. novo-ulmi* and the growth rings before and after inoculation were assessed. The most tolerant seedlings formed large earlywood vessels. Since wide earlywood vessels would favour pathogen dispersion and cavitation, the tolerance of these individuals probably relied on resistance mechanisms other than anatomical. In the literature, relationships between xylem diameter and tree tolerance are not always found. After inoculation, a marked reduction of vessel size was observed, and most trees formed a tangential band of large vessels in the latewood, possibly as response to the dysfunction of earlywood vessels. Vessel occlusion by gums and tyloses, which contribute to hydraulic dysfunction, was more frequent in the more susceptible trees. However, latewood with narrow vessels and low abundance of vessels possibly allowing pathogen compartmentalization was more abundant in tolerant trees. In one progeny, the susceptibility to DED showed mean values similar to the mother tree, which suggests maternal effects in disease tolerance. Latewood vessel area and other anatomical traits of seedlings were similar to those observed in their mother trees. Results suggest that in offspring of tolerant trees (i) earlywood xylem anatomy is not as determinant of tolerance as the latewood, and (ii) inheritance of anatomical traits seems to be maternal.

## 76. Chestnut habitat restoration via the biological control of *Cryphonectria parasitica* in protected areas of Tismana (Romania)

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*Cryphonectria parasitica* has destroyed the American chestnut (*Castanea dentata*) habitats and produced severe degradation in the European chestnut (*Castanea sativa*) habitats. In Romania, there are just two small regions where chestnut creates forest habitats, and both of them have been severely degraded by the chestnut blight. All the old forests were killed by the Asian pathogen. The biological control of *C. parasitica* has proved to be generally successful in Maramures region (NW Romania) between 2005 and 2008. The same method was used to save the chestnut habitats of Tismana zone (South Carpathians), and the results are presented in the present paper. Treatments were done between 2013 and 2017. Intense inoculations (on almost all the sprout groups) have induced rapid recovery of the health status of the young regeneration of chestnut due to the high compatibility of EU12 strain with CVH1 virus. We also set up new chestnut plantations in order to partially recover the habitat lost area caused by the combined effects of *C. parasitica* and competition with European beech, common hornbeam, etc. The sprouts were severely damaged by red deer in two treated parcels, but the inoculations were not influenced even the bark-stripping occurred in the next winter after the treatment. In older stands we could not perform the treatment due to height of the cankers, therefore just few suitable trees were inoculated to spread the virus in the whole area; in these habitats the chestnut status is still uncertain. Eventually the virulence of the pathogen has significantly diminished, and the chestnut habitats of Tismana have improved their health status in young stands.

**Acknowledgement.** This work was supported by the LIFE+11NAT/RO/825 project.

**Keywords:** chestnut blight, EU12, CHV1, habitat recovery.

## 77. Infection of *Fraxinus excelsior* by *Hymenoscyphus fraxineus* in pure and mixed young stands in Latvia

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Since 1990<sup>th</sup> dieback of ash, caused by *Hymenoscyphus fraxineus*, has been observed in Latvia. Previous studies in Latvia and elsewhere have indicated, that health status of ash might be better in mixed compare to pure stands of this tree species. However, there has been limited information on impact of tree species mixture on infection of young ashes (advance regeneration). Repeated inventory (in august of 2015 and 2017) of health (determined by 5 grades scale) of advance regeneration of ashes (c.a. 3000 trees) had been carried out in 35 randomly selected young (26-36 years) ash-dominated (>30%) stands across Latvia based on methodology by Lygis et al., 2014.

Statistically significant differences in health status of advance regeneration of ash trees was observed in pure and mixed stands in both years of the inventory: ashes were healthier in mixed stands. They were also significantly healthier in 2017 in comparison to 2015 (average grade 2.07 and 2.47, respectively), while differences between the forest site types remained: best health in 2017 and improvement of it was observed in forests on drained minerals soils (average grade 2.64 in 2015 and 1.72 in 2017). Health status of ash trees was different depending on the tree species in admixture: it was best in stand mixed with black and grey alder and worst in stands with Norway spruce. Mixture with Norway spruce most likely will form a pure spruce stands in future, but in stands mixed with silver birch health status of ash was declining between the inventories, suggesting additional impact of the disease and un-predictable survival of advance regeneration of ash in future.

**Keywords:** *Fraxinus excelsior*, *Hymenoscyphus*, ash dieback, mixed stands

**Acknowledgement.** Study was carried out in ERA-NET SUMFOREST project “Mixed species forest management. Lowering risk, increasing resilience (REFORM)”.

## 78. The presence of *Rhodotorula* sp. in *Ulmus minor* plantlets moderates the response to *Ophiostoma novo-ulmi* in roots of tolerant clones

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Dutch elm disease (DED) is caused by the fungus *Ophiostoma novo-ulmi* and is one of the most devastating forest diseases of the last century. However, there are some elm genotypes able to tolerate this disease. Although some mechanisms involved in the tolerance have been already characterized, the molecular basis of the tolerance is still unknown. In addition, the role of the tree microbiome in elm tolerance to DED is poorly known. In this work, we aimed to observe the molecular responses of tolerant and susceptible *Ulmus minor* genotypes after the inoculation of either the pathogen or one endophytic fungus of the genus *Rhodotorula* which was found to be more abundantly present in tolerant than susceptible genotypes. We worked with 1 month-old plantlets grown *in vitro*. The roots were submerged in a solution of spores of each fungus and later the plants were grown for a week preserving the sterility. The expression of 7 genes related to local defense responses was analyzed in the roots. Both fungi produced a strong overexpression of *phenylalanine ammonia lyase* (*PAL*) and *pathogenesis-related protein 4* (*PR4*) genes. However the pathogen induced a higher response than the endophyte. In a second experiment, we explored the ability of the endophyte to induce resistance in the elm against *O. novo-ulmi*. Then, a new treatment included the inoculation with *Rhodotorula* prior to the pathogen inoculation. In comparison with the single inoculation of *O. novo-ulmi*, the double inoculation showed a reduction of the *PAL* overexpression in tolerant genotypes, while no effect or increased expression was observed in the susceptible ones. These results suggest a relationship between the tolerance to DED and the association of certain endophytes to some elm genotypes.

## 79. Control of oxidative stress as a tolerance mechanism in *Ulmus minor* against *Ophiostoma novo-ulmi*

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The high virulence of the Dutch elm disease (DED) pathogen, *Ophiostoma novo-ulmi*, has decimated European and North American elm populations during the last century. The number of native resistant elm clones available on the market is scarce, and the defense mechanisms that render a tree tolerant or resistant to the disease are not well understood. In this work we cultivated *in vitro* *Ulmus minor* plants with the aim to: i) study the pathogen dispersion rate in two clones of contrasting susceptibility level to DED, and ii) characterize plant stress responses after *O. novo-ulmi* inoculation. At 1, 3, 7, 14 and 21 days after inoculation (dai) we monitored: pathogen spread within the plant, plant oxidative stress (lipid peroxidation), content of phenolic compounds, level of chlorophylls, and plant growth. We also performed an anatomical study of the xylem to evaluate the possible role of conduit size on susceptibility. The rate of pathogen dispersal was similar in both clones, suggesting that *tolerance* rather than *resistance* mechanisms operate in the low susceptible clone. Xylem anatomy was also similar in both clones, and therefore differences in the response to the pathogen were not attributable to anatomical factors. After pathogen inoculation, the tolerant clone showed a marked increase in lipid peroxidation at 1 dai, but afterwards the levels decreased to values of control plants. The susceptible clone, on the contrary, showed enhanced values of lipid peroxidation at 1, 3, 14 and 21 dai. A peak in total phenolic compounds was detected in the tolerant clone at 3 dai, while no significant changes were observed in the susceptible clone. The susceptible but not the tolerant plantlets suffered a significant delay in apical growth and a decrease in chlorophyll content at 21 dai. The results suggest that the tolerant clone, in spite of being widely colonized by the pathogen, rapidly controlled the induced oxidative burst by producing antioxidant compounds. The susceptible clone maintained a high oxidative stress level during a long period after inoculation, which possibly led to cell damage, cessation of growth and reduced chlorophyll content. Oxidative stress homeostasis thus appears as a factor that can contribute to elm tolerance to DED. Furthermore, the *in vitro* system used in the experiment arises as plausible early-screening method of elm tolerance.

## 80. The LIFE+ ELM project “Elms Alive” for the restoration of *Ulmus minor* and *Ulmus laevis* in Spain

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The degradation of European elm stands due to the negative impact of human-induced changes in riparian ecosystems and the emergence of the highly aggressive Dutch elm disease (DED) pathogens prompted several elm breeding programs. In Spain, seven *Ulmus minor* clones tolerant to DED were recently selected and the small and fragmented *Ulmus laevis* populations were proven to be native. With this background, the LIFE+ ELM project (2014-2019; slogan “elms alive”; [www.olmosvivos.es](http://www.olmosvivos.es)) has the overall objectives of monitoring acclimation of selected clones to riparian habitats presumed to be suitable for reintroduction (within the Tagus river basin), and to gain experience and ecological knowledge for future implementation of large scale elm restoration activities. It also intends to recover the use of elms as an ornamental shade tree in urban landscaping. By now, 11,156 individuals have been planted in forest and urban green areas, representing 67% of the total planned plantations. Plant material consists of clonal copies of the seven *Ulmus minor* clones obtained by micropropagation and *U. laevis* seedlings from two relict populations in the restoration area. The limited attractiveness of this last species for the elm bark beetles points to a low risk of DED damage in *U. laevis*. Due to the lack of earlier restoration attempts, it will be necessary to evaluate factors such as distance to the river, elevation, soil humidity, and incidence of pests, diseases and herbivory to form conclusions about the adaptability and survival of each elm genotype and species. To this end, experimental plots have been established in the restoration areas and preliminary results show a significant genotype x environment interaction. The ultimate goal is for the populations of both elm species to reach sexual maturity so they can contribute to gene flow and conservation of native genetic resources. Another key aim of LIFE + ELM is to transfer the knowledge and experience gained in the project to other players in forest restoration and the general public.

**Acknowledgement.** Funding: LIFE13 BIO/ES/000556 LIFE+ ELM.

## 81. Ash DieBack and Dutch Elm Disease: current situation and prospects in Slovenia

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Ash dieback has been present in Slovenia since 2006, and Dutch elm disease since 1929. We have evaluated their current situation in Slovenia based on sanitary felling. Sanitary felling of ash has risen exponentially from 2009 to 2017. In 2017, 76,101 m<sup>3</sup> of ash or 69% of total felling was due to ash dieback, which represents 2% of ash wood stock. Geographically more damaged forests (0.5–2.1% of wood stock) were in the eastern part of Slovenia. We suspect that the sanitary felling of ash will escalate and follow the exponential trend to some level, and wood stock of ash will drop by 20–40% in next 10 years. From 1995 to 2013, between 51 and 59% of elm was felled due to Dutch elm disease. Sanitary felling was disturbed between 2014 and 2016 because of the catastrophic ice damage that happened in 2014. The most damaged areas are in the southern part of Slovenia, where 11–75% of elm wood stock was damaged due to Dutch elm disease. However, in the most cases, only up to 2% of elm wood stock was sanitarily felled. The prospect for elms is becoming better over the years as sanitary felling due to Dutch elm disease is slightly lower, and the total wood stock of elms has risen from 2007 to 2013 and remained on that level since then. Therefore, we believe that elms are slowly recovering from Dutch elm disease. However, other damaging factors threaten elms.

**Keywords:** *Hymenoscyphus fraxineus*, *Chalara fraxinea*, *Ophiostoma ulmi*, *Ophiostoma novo-ulmi*, sanitary felling, *Fraxinus*, *Ulmus*

**Acknowledgements.** We thank Public Forestry Service, Ministry of Agriculture, Forestry and Food, Slovenian Forestry Service, and Slovenian Research Agency (research core funding No. P4-0107).



## 82. Physiological response and resilience to flooding of five *Ulmus minor* genotypes tolerant to Dutch Elm Disease

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The Spanish elm breeding program selected five *Ulmus minor* genotypes tolerant to Dutch elm disease (V-AD2, M-DV5, M-DV2.3, M-MJ1 and M-RT1.5) with the main objective of recovering the Spanish elm stands. In future restoration actions, these genotypes will be planted in areas prone to flooding events. To clarify the tolerance of these genotypes to flooding, and their resilience during the post-flooding period, a simulation flooding experiment was carried out using 1-year-old potted plants. Flood stress was maintained during five weeks (mid-June – early July) followed by seven weeks of a normal watering (mid-July – early September). Physiological measurements of plants were performed at the end of both periods. Gas exchange variables such as net photosynthetic rate (P<sub>n</sub>), stomatal conductance (g<sub>s</sub>) and transpiration rate (E) were significantly reduced in all genotypes, as well as quantum yield of photosystem II (PhiPSII) and electron transport rate (ETR). However, xylem water potential remained unaltered, suggesting that stomatal closure could have been produced by chemical rather than hydraulic signals. Despite a reduction on gas exchange variables, the concentration of soluble sugars in leaves was unchanged, and increased in the roots. The survival rate was high, as only M-DV2.3 and M-RT1.5 genotypes showed 10-15 % of mortality due to flooding. Also, all genotypes were able to successfully recover physiological functions after seven weeks of normal watering. The results suggest that tested genotypes of *Ulmus minor* are relatively resistant and resilient to flood stress.

## 83. The assessment of inoculum potential of *Armillaria gallica* in oak stands

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### Introduction

*Armillaria* spp. and *Desarmillaria* spp. cause the most important tree diseases. Disease occurs in forestry, horticulture, agriculture, and in parks and gardens. *Armillaria gallica* is the most common species in oak stands and seems to have an important role in oak decline. The species attacks mostly the most susceptible trees weakened by stress factors e.g. water deficiency.

### Materials and methods

The study was conducted on 12 **pedunculate** oak (*Quercus robur* L.) stands in age classes V, VIII and IX (four stands in each age class, 25 oaks on each plot) located in the Krotoszyn Forest District (N 51° 46'6.805", E 17°37'14.124"), which is the one of the most valuable oak complexes in Poland. The inoculum potential of *A. gallica* in oak stands was assessed using four methods which characterize inoculum density:

1. Bait method: 30 aspen poles were buried in soil of each plot and after seven months the dry mass of rhizomorphs developed on the poles was assessed.
2. Small pit method: 10 pits (30 x 30 x 30 cm) were dug across each plot, 2 m apart, and rhizomorphs present in the pits were counted.
3. Grid method: epiphytic rhizomorphs present on root collars were counted within a grid (5.5 x 5.5 cm black plastic sheet with 25 holes, 5 mm diam., placed 1 cm apart; Marçais & Caël 2006). The area of a root collar assessed represented 20% of the root collar area of 300 trees growing on the experimental plots.
4. The presence of rhizomorphs in soil around trees: dry mass of rhizomorphs from soil collected at a 30 cm radius and 30 cm depth.

The health of all trees in each combination (300 trees) was assessed on the basis of the condition of the tree (defoliation, death of twigs and branches, decay of trunk and branches, presence of fruit-bodies of wood-decay fungi). The health was described as good, moderate or bad.

### Results

On average 100%, 75% and 50% of aspen poles were colonized by rhizomorphs in stands of age classes V, VIII and IX. The dry mass of rhizomorphs in age classes V, VIII and IX accounted for 73.7%, 12.9% and 13.4% of the total dry mass from all baits. Rhizomorphs present in small pits in stands of age classes V, VIII and IX accounted for 47%, 30% and 23% of the total number of rhizomorphs in all pits. Rhizomorphs spotted in the grid holes in stands of age classes V, VIII and IX accounted for 66%, 24% and 10% of the total number of rhizomorphs in all plots. The number of rhizomorphs increased with decreasing health of the trees; they were present on 33%, 30% and 45% of trees with good, moderate or bad health. Each 10 cm<sup>2</sup> area of root collar in age classes V, VIII and IX was colonized by 3.38, 0.97 and

0.5 rhizomorphs. Dry mass of rhizomorphs around trees in stands of age classes V, VIII and IX was 56.2%, 30.1% and 13.7% of the total dry mass from all plots. Dry mass of rhizomorphs around trees with good, moderate or bad health accounted for 28%, 32.8% and 39.2% of the total number of rhizomorphs in all plots.

The estimated dry mass of rhizomorphs in 1 ha in stands of age classes V, VIII and IX was 1103 kg (859-1271 kg), 417 kg (199 -809 kg) and 188 kg (0-479 kg). There was a positive correlation between the dry mass of rhizomorphs colonizing aspen poles and the number of rhizomorphs present on root collars, and between the latter and the number of rhizomorphs in small pits or dry mass of rhizomorphs in soil around trees.

### **Conclusions**

- The density of rhizomorphs in soil decreases with increasing age of the stand and increasing distance from the trees. This may affect (i.e. decrease) the contribution of *Armillaria* to oak decline.
- The bait and small-pit methods can be used to determine inoculum potential of *Armillaria gallica* in oak stands.
- The number of rhizomorphs on the root collar of oaks reflects their activity in the soil around the trees.
- The health of oak reflects the *Armillaria* inoculum density around the trees.

### **References**

Marçais B., Caël O. 2006. Spatial pattern of the density of *Armillaria* epiphytic rhizomorphs on tree collar in an oak stand. *Forest Pathology* (36): 32–40.

#### **84. Analysis of genetic and phenotypic diversity provide insights on the epidemiology of *Laetiporus sulphureus* in Sicilian carob trees**

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*Laetiporus sulphureus* (Bull) Murrill. is a forest pathogen causing brown cubical heart rot in a broad range of host trees. Despite its importance, studies aimed at understanding the epidemiology of *L. sulphureus* in specific areas or hosts are lacking. In this study, a survey conducted with molecular analysis was performed on 70 carob (*Ceratonia siliqua* L.) trees in the South West of Sicily to determine the incidence of the pathogen. A phylogenetic analysis of internal transcribed spacer (ITS) sequences from isolates obtained from fruiting bodies collected from carob orchards and other tree species indicated that all of them belonged to the cluster E of *Laetiporus* taxonomy. Thanks to the availability of the genome draft of *L. sulphureus*, molecular markers based on Simple Sequence Repeats (SSR) were developed and isolates were genotyped through High Resolution Melting (HRM) analysis. An *in vitro* growth assay was performed, and data from genotypic and phenotypic diversity were compared, to assess the occurrence of possible correlation. Results of population genetic analysis showed high gene diversity (0.581), absence of correlation between fungal genotype and host plants, and significant correlation between spatial and genetic distance. These results suggest for *L. sulphureus* a high risk of transmission from a tree species to the others. In addition, results strongly support the hypothesis that spread of the fungus is mainly occurring by means of basidiospores.

## **Field excursion LifeELMIAS konferens 31 August on Gotland**

### **Program**

<i>Field excursion 31 August</i>		<b>In charge:</b>
<b>12.00</b>	<i>Picking up at Wisby Strand conference hall Departure to Allkvie meadow (Endre)</i>	
<b>12.30 – 13.45</b>	<b>Lunch</b> (30 min) <i>Allekvie meadow;</i> <ul style="list-style-type: none"> <li>• <i>Information about a wooded hay meadow of Gotland, history, management and future challenges (30 min)</i></li> <li>• <i>Actions done to fight DED (30 min)</i></li> <li>• <i>Restoration actions (15 min)</i></li> </ul>	<i>Ingrid Thomasson Astrid Dale Karin Wågström</i>
<b>13.45</b>	<i>Departure to Seed plantation, Barlingbo</i>	
<b>14.00 – 14.15</b>	<i>Ashseed plantation, Barlingbo</i>	<i>Rimvydas Vasaitis Audrius Menkis Jan Stenlid</i>
<b>14.15</b>	<i>Departure to Klosteränge (Halla)</i>	
<b>14.35 – 15.45</b>	<i>Review of implemented measures in Klosteränge: (70 min)</i> <ul style="list-style-type: none"> <li>• <i>Fencing plants of ashes and oaks</i></li> <li>• <i>Veteranisation</i></li> <li>• <i>Restoration actions; clearing around broadleaved trees and clearing of annex 1 habitat</i></li> </ul>	<i>Ingrid Thomasson Vikki Bengtsson</i>
<b>15.45</b>	<i>Departure to Högklint, Visby</i>	
<b>16.15 – 16.45</b>	<i>Högklint, Visby Close of konferens - traveling back to Visby, arrives about 17.00</i>	

## Exkursionspunkt Allkvie änge

1. **Gotlandsänget, historik, skötsel och hot.** (Ingrid Thomasson, Astrid Dale)
2. **Genomförda bekämpningsåtgärder mot almsjuka** (almsjuka träd, avverkning, stubbehandling mm) (*Karin Wågström mfl*)
3. **genomförda skötselåtgärder i Allkvie änge** (*Ingrid Thomasson*)



## Exkursionspunkt Klosteränge

Uppföljning av skötselåtgärder i Natura 2000-områden (Ingrid Thomasson, Vikki Bengtsson,)

- Restaurering, friställning och veteranisering



## Excursion route Gotland

Förslag på körrutt, LifeELMIAS konferens 31 augusti 2018



1. Allkvie änge
2. Seed plantagen, Barlingbo
3. Klosteränge
4. Höglint