Situation with Ash in France: stand characteristics, health condition, ongoing work and research needs

Benoit Marçais, Claude Husson, Samuel Lemonnier, Arnaud A. Dowkiw

COST ACTION FP1103 FRAXBACK 1st MC/WG Meeting, November 13-14th, Radisson Blu Hotel, Vilnius, Lithuania

"Situation with Ash in MY COUNTRY: stand characteristics, health condition, ongoing work and research needs"
("my country" to be inserted into respective presentation title, see below)

MEETING PROGRAM

November 12th: arrival and accommodation;

November 13th:

08:30 – 09:00, registration and signing the attendance list;

09:00 – 10:40, Session 1 *(Chair: Rimvys VASAITIS)*

09:00 – 09:20, general information: Rimvys VASAITIS and Cassia AZEVEDO;
09:20 – 10:00, presentations of Working Groups by WG Leaders:

- WG1 "Pathogen": Barbara SCHULZ; (15 MIN)
- WG2 "Host": Thomas KIRISITS; (15 MIN)
- WG3 "Silviculture": Jens Peter SKOVSGAARD; (10 MIN)
- WG4 "STSMs, dissemination and research needs": Anna RYTKÖNEN (10 MIN);

10:10 – 10:25, **AUSTRIA:** Thomas CECH, Christian FREINSCHLAG, Berthold HEINZE, Thomas KIRISITS, Heino KONRAD, Katharina KRAUTLER, Peter KRITSCH, Stephanie STEINBOECK, Marcela VANLOO;
10:25 – 10:40, **BELGIUM:** Anne CHANDELIER;

10:40 – 11:10, BREAK (coffee/tea & snacks could be bought in a lobby bar);

11:10 – 12:45, Session 2 *(Chair: Barbara SCHULZ)*

11:10 – 11:20, **BULGARIA:** Margarita GEORGIEVA, Ivaylo TSVETKOV;
11:20 – 11:35, **CZECHIA:** Milon DVORAK, Lida HAVRDOVA, Libor JANKOVSKY, Zdenka PROCHAZKOVA, Petr MARSIK;
11:35 – 11:50, **CROATIA:** Danko DIMINIC, Davorin KAJBA;
11:50 – 12:05, **DENMARK:** Lea Vig MCKINNEY, Lene Rostgaard NIELSEN, Ditte C. OLRIK, Iben Margrete THOMSEN;
12:05 – 12:20, **ESTONIA:** Rein DRENKHAN, Tiit MAATEN;
12:20 – 12:35, **FINLAND:** Arja LILJA, Anna RYTKÖNEN, Anne UIMARI;
12:35 – 12:45, **GREECE:** Dionisios GAITANIS, Ioannis MELIADIS, Gavriil SPYROGLOU;

12:45 – 14:00, LUNCH (buffee at hotel restaurant, 13 €);

14:00 – 15:25, Session 3 *(Chair: Anna RYTKÖNEN)*

14:00 – 14:15, **FRANCE:** Arnaud DOWKIW, Claude HUSSON, Benoit MARCAIS; Samuel LEMONNIER;

14:15 – 14:30, **GERMANY:** Rasmus ENDERLE, Corina JUNKER, Berthold METZLER, Barbara SCHULZ, Heike LENZ;
14:30 – 14:45, **HUNGARY:** Gergely JANIK, Andras KOLTAY;
14:45 – 14:55, **IRELAND:** Gerry DOUGLAS, Cathal RYAN;
14:55 – 15:10, **LATVIA:** Natalija ARHIPOVA, Indulis BRAUNERS, Talis GAITNIEKS, Dainis RUNGIS;
15:10 – 15:25, **LITHUANIA:** Alma GUSTIENE, Vaidotas LYGIS, Diana MARCIULYNIENE, Alfas PLIURA, Vytautas SUCHOCKAS;

15:25 – 16:00, BREAK;

16:00 – 17:20, Session 4 *(Chair: Thomas KIRISITS)*

16:00 – 16:10, **ITALY:** Nicola LUCHI, Lucio MONTECCHIO, Alberto SANTINI;
16:10 – 16:25, **NETHERLANDS:** Sven DE VRIES, Jitze KOPINGA, Fons VAN KUIK;
16:25 – 16:40, **NORWAY:** Halvor SOLHEIM, Mari-Mette TOLLEFSRUD;
16:40 – 16:55, **POLAND:** Wojciech KRAJ, Justyna NOWAKOWSKA, Tomasz OSZAKO, Artur PACIA, Dorota DOBROWOLSKA;
16:55 – 17:10, **ROMANIA:** Dan ALDEA, Danut CHIRA, Valeriu-Norocel NICOLESCU;

17:10 – 18:10, POSTER SESSION;

18:10 –19:00, meetings of participants with the leaders of respective Working Groups 1-4 (venues to be announced)

November 14th:

09:00 – 10:15, Session 5 *(Chair: Jens Peter SKOVSGAARD)*

09:00 – 09:15, **RUSSIA:** Dmitry MUSOLIN, Andrey SELIKHOVKIN;
09:15 – 09:25, **SERBIA:** Nenad KECA, Ivan MILENKOVIC;
09:25 – 09:40, **SLOVAKIA:** Andrej KUNCA, Valeria LONGAUEROVA, Miriam MALOVA, Katarina ADAMCIKOVA;
09:40 – 10:05, **SLOVENIA:** Tine HAUPTMAN, Nikica OGRIS, Marjana WESTERGREN;
10:05 – 10:15, **SPAIN:** Julio J. DIEZ CASERO;

10:15 – 11:15, BREAK, and eventual check-out;

11:15 – 12:20, Session 6 *(Chair: Arnaud DOWKIW)*

11:15 – 11:30, **SWEDEN:** Stina BENGTSSON, Michelle CLEARY, Haleh HAYATGHEIBI, Jens Peter SKOVSGAARD, Jan STENLID, Rimvys VASAITIS, Johanna WITZELL, Pia BARKLUND;
11:30 – 11:45, **SWITZERLAND:** Matthias DOBBERTIN, Andrin GROSS, Daniel RIGLING;
11:45 – 11:55, **TURKEY:** Tugba DOGMUS LEHTIJARVI, Asko LEHTIJARVI;
11:55 – 12:10, **UKRAINE:** Iryna MATSIAKH, Kateryna DAVYDENKO;
12:10 – 12:20, **UNITED KINGDOM:** Steven HENDRY, Nick MAINPRIZE, John WEIR;

12:20 – 13:30, **MANAGEMENT COMMITTEE MEETING:** agenda attached separately *(Chair: Rimvys VASAITIS)*

13:30 – closing the meeting ...

EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

List of participants of the 1st FRAXBACK WG/MC Meeting, 13th-14th of November, Hotel Radisson Blu Lietuva, Vilnius, Lithuania (in alphabetical order)

1. Katarina Adamcikova; *Slovakia*
2. Dan ALDEA; *Romania*
3. Natalija ARHIPOVA; *Latvia*
4. Cassia AZEVEDO; *Belgium*
5. Pia BARKLUND; *Sweden*
6. Stina BENGTSSON; *Sweden*
7. Indulis BRAUNERS; *Latvia*
8. Thomas L. CECH; *Austria*
9. Anne CHANDELIER; *Belgium*
10. Danut CHIRA; *Romania*
11. Michelle CLEARY; *Sweden*
12. Kateryna DAVYDENKO; *Ukraine*
13. Sven DE VRIES; *Netherlands*
14. Julio J. DIEZ CASERO; *Spain*
15. Danko DIMINIC; *Croatia*
16. Matthias DOBBERTIN; *Switzerland*
17. Dorota DOBROWOLSKA; *Poland*
18. Tugba DOGMUS LEHTIJARVI; *Turkey*
19. Gerry DOUGLAS; *Ireland*
20. Arnaud DOWKIW; *France*
21. Rein DRENKHAN; *Estonia*
22. Milon DVORAK; *Czechia*
23. Rasmus ENDERLE; *Germany*
24. Christian FREINSCHLAG; *Austria*
25. Dionisios GAITANIS; *Greece*
26. Talis GAITNIEKS; *Latvia*
27. Margarita GEORGIEVA; *Bulgaria*
28. Andrin GROSS; *Switzerland*
29. Alma GUSTIENE; *Lithuania*
30. Tine HAUPTMAN; *Slovenia*
31. Lida HAVRDOVA; *Czechia*
32. Haleh HAYATGHEIBI; *Sweden*
33. Berthold HEINZE; *Austria*
34. Steven HENDRY; *United Kingdom*
35. Claude HUSSON; *France*
36. Gergely JANIK; *Hungary*
37. Libor JANKOVSKY; *Czechia*
38. Corina JUNKER; *Germany*
39. Davorin KAJBA; *Croatia*
40. Nenad KECA; *Serbia*

41. Thomas KIRISITS; *Austria*
42. Andras KOLTAY; *Hungary*
43. Heino KONRAD; *Austria*
44. Jitze KOPINGA; *Netherlands*
45. Wojciech KRAJ; *Poland*
46. Katharina KRAUTLER; *Austria*
47. Peter KRITSCH; *Austria*
48. Andrej KUNCA; *Slovakia*
49. Asko LEHTIJARVI; *Turkey*
50. Samuel LEMONNIER; *France*
51. Heike LENZ; *Germany*
52. Arja LILJA; *Finland*
53. Valeria LONGAUEROVA; *Slovakia*
54. Nicola LUCHI; *Italy*
55. Vaidas LYGIS; *Lithuania*
56. Tiit MAATEN; *Estonia*
57. Nick MAINPRIZE; *United Kingdom*
58. Miriam MALOVA; *Slovakia*
59. Benoit MARCAIS; *France*
60. Petr MARSIK; *Czechia*
61. Diana MARCIULYNIENE; *Lithuania*
62. Iryna MATSIAKH; *Ukraine*
63. Lea V. MCKINNEY; *Denmark*
64. Ioannis MELIADIS; *Greece*
65. Berthold METZLER; *Germany*
66. Ivan MILENKOVIC; *Serbia*
67. Lucio MONTECCHIO; *Italy*
68. Dmitry MUSOLIN; *Russia*
69. Valeriu N. NICOLESCU; *Romania*
70. Lene R. NIELSEN; *Denmark*
71. Justyna NOWAKOWSKA; *Poland*
72. Nikica OGRIS; *Slovenia*
73. Ditte C. OLRIK; *Denmark*
74. Tomasz OSZAKO; *Poland*
75. Artur PACIA; *Poland*
76. Alfas PLIURA; *Lithuania*
77. Zdenka PROCHAZKOVA; *Czechia*
78. Daniel RIGLING; *Switzerland*
79. Dainis RUNGIS; *Latvia*
80. Cathal RYAN; *Ireland*
81. Anna RYTKONEN; *Finland*
82. Alberto SANTINI; *Italy*
83. Barbara SCHULZ; *Germany*
84. Andrey SELIKHOVKIN; *Russia*
85. Jens P. SKOVSGAARD; *Sweden*

86. Halvor SOLHEIM; *Norway*
87. Kiril SOTIROVSKI; *F.Y.R. Macedonia*
88. Gavriil SPYROGLOU; *Greece*
89. Stephanie STEINBOECK; *Austria*
90. Jan STENLID; *Sweden*
91. Vytautas SUCHOCKAS; *Lithuania*
92. Iben M. THOMSEN; *Denmark*
93. Mari-Mette TOLLEFSRUD; *Norway*
94. Ivaylo TSVETKOV; *Bulgaria*
95. Anne UIMARI, *Finland*
96. Fons VAN KUIK; *Netherlands*
97. Marcela VANLOO; *Austria*
98. Rimvys VASAITIS; *Sweden*
99. John WEIR; *United Kingdom*
100. Marjana WESTERGREN; *Slovenia*
101. Matt CASTLE; *United Kingdom*
102. Gilli THORP; *United Kingdom*

ABSTRACTS OF COUNTRY PRESENTATIONS

Ash and ash dieback in Austria: Occurrence and importance of ash, situation of the disease, past and ongoing work and research needs

Thomas Kirisits[1,3], Thomas L. Cech[2], Christian Freinschlag[1], Berthold Heinze[3], Heino Konrad[3], Katharina Kräutler[1], Peter Kritsch[1], Stephanie Steinböck[1], Marcela van Loo[4]

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Occurrence and importance of ash in Austria
Three species of ash (*Fraxinus*) occur naturally in Austria. While European or common ash (*Fraxinus excelsior*) is widespread and abundant on appropriate sites in many parts of the country, the two other species, narrow-leaved ash (*Fraxinus angustifolia* subsp. *danubialis*) and flowering ash (*Fraxinus ornus*), reach edges of their distribution ranges in Austria and are therefore rare. Green ash (*Fraxinus pennsylvanica*) is the only non-native ash species of certain importance in forests.

In the presentation of the results of the Austrian Forest Inventory (AFI, www.waldinventur.at), the four ash species mentioned above are not differentiated, but

F. excelsior has the vast majority of the share of *Fraxinus* spp. In the AFI period 2007-2009 ash had a share of 3.1 % (based on the number of trees) and 2.1 % (based on the volume of timber growing) in managed forests. Depending whether the number of trees or the volume of timber growing are considered, ash is the second to third most frequent group of broadleaved tree species in managed forests in Austria. Common ash, although not among the major timber species, is of considerable socio-economic and ecological importance in Austria. The two other native ash species are of local importance, particularly also from the perspective of nature conservation. *Fraxinus pennsylvanica* has become naturalized in some areas in eastern Austria, where it is of concern as alien invasive species. Other non-native ash species from North America and Asia are only very rarely seen in arboreta and botanical gardens in Austria.

History and importance of ash dieback in Austria

The first unambiguous observations of symptoms of ash dieback (shoot dieback, necrotic lesions in the bark, wood discoloration) were made in 2005, at a few sites in the provinces of Lower Austria, Upper Austria and Salzburg, on young *F. excelsior* trees in afforestations. In 2005, massive early leaf shedding of ash trees, occurring already in late August and early September, was observed in parts of the provinces of Lower and Upper Austria. The same happened 2006 in parts of Styria. Originally, this phenomenon was thought to be caused by powdery mildews, but as *Hymenoscyphus pseudoalbidus* also causes leaf symptoms, this was likely the first widespread indication of ash dieback. In subsequent years, obvious early leaf shedding occurred regularly in various parts of the country, particularly in the Alps. Intensive dieback of shoots, twigs and branches of ash trees of all ages occurred for the first time in 2006 over wide areas in the provinces of Lower and Upper Austria and in 2007 in Styria. In subsequent years the disease occurred steadily at new places and gradually increased in incidence and severity.

Although the spread of the ash dieback epidemic was not followed in much detail, observations by experts and reports by practitioners suggest that the disease emerged later in western, southern and south-eastern parts of the country, compared to eastern and northern Austria. In 2008 symptoms were observed in all Austrian provinces, though the reports from Tyrol and Vorarlberg were made by practitioners and not by experts and are therefore somewhat ambiguous (Table 1). At least by 2010 the disease has likely been present in all political districts of the country. A survey in July 2010 in parts of Carinthia and Eastern Tyrol indicated that some localized areas in these provinces were still free of ash dieback. By now, there are likely no disease-free areas any more.

As in other countries, the etiology of ash dieback was initially poorly understood and its causes were debated. *Hymenoscyphus pseudoalbidus* (at that time only known in its anamorphic stage, *Chalara fraxinea*) was for the first time isolated in Austria in June 2007. Subsequent surveys showed that the pathogen is widespread in the country and from June 2007 until June 2011 it was detected in all Austrian provinces, at a total of 141 localities (Table 1). When isolations were made from shoots, twigs and stems showing early symptoms of disease, *C. fraxinea* was the most consistently and most frequently isolated fungus. Its pathogenicity was confirmed in a number of inoculation experiments on various hosts. Besides *F. excelsior* and its variety '*Pendula*', naturally infected hosts include *F. angustifolia* and *F. pennsylvanica*. No natural infection were, however, found on *F. ornus*, and we suppose that this species in not a host of the ash dieback pathogen.

Ash dieback is presently the most important disease of hardwood trees in Austria and it seriously questions the future use of *F. excelsior* as economically and ecologically valuable noble hardwood species. Particularly, natural regeneration, seedlings and saplings are severely damaged. Mortality is also fairly common in trees up to 30 years and is occasionally observed even in old ash trees. Necrotic lesions and wood discoloration on the root collar and lower stem, recently reported to be caused by *H. pseudoalbidus*, appear to occur frequently also in Austria. Even if infected ash trees survive, their growth and their quality with regard to timber production can be severely impaired, due to forking and wood discolorations.

Observations suggest that the intensity of ash dieback varies considerably in various parts of Austria. Ash dieback appears to be most severe in the Northern Limestone Alps and on moist sites (e. g. in glens), while the impact of the disease is slightly less on dry sites, particularly in eastern and north-eastern Austria. However, it remains to be seen, whether these differences offer perspectives for disease management and silviculture with ash. As in many other countries, individual ash trees in severely affected stands are only slightly damaged, indicating that they may possess relatively high resistance levels to the ash dieback pathogen.

Past and ongoing work

Since 2007 research and dissemination activities concerning ash dieback have been conducted in Austria, by BFW and IFFF-BOKU. A brief description of the activities follows below:

- The intensity of ash dieback on mature ash trees has been monitored on 14 permanent plots from 2007 to 2011 in Lower Austria (see the accompanying poster presentation by Keßler at al.).
- Ash dieback has been included in the ‘Documentation of forest damage factors’, a monitoring system conducted every year that is based on expert opinions and appraisals of staff of the district forest authorities.
- As already mentioned above, a survey of *H. pseudoalbidus* (*C. fraxinea*) was conducted from June 2007 to June 2011, documenting the widespread and common occurrence of the pathogen in Austria (Table 1); see e. g. the accompanying poster presentation by Kirisits et al. on the work in forest nurseries.
- The phenology of sporulation of *H. pseudoalbidus* was investigated using spore traps and by observing ascocarps periodically.
- Inoculation and germination studies with asexual spores of *C. fraxinea* indicated that they are likely not infectious, but probably serve as spermatia.
- The symptomatology of the disease was studied based on observations, fungal isolations and new findings regarding the biology of the ash dieback pathogen; in 2009 a hypothetic disease cycle of ash dieback was proposed.
- Numerous wound inoculation experiments with potted seedlings were conducted; Kochs postulates were fulfilled to conclude that *H. pseudoalbidus* is associated with ash dieback on *F. excelsior*, *F. angustifolia* and *F. pennsylvanica*. *Hymenoscyphus pseudoalbidus* also caused symptoms on *F. ornus*, although natural infections have so far not been detected on this ash species.
- In 2012, a stem inoculation experiment on potted seedlings was initiated to examine the role of water stress in the susceptibility of *F. excelsior* to *H. pseudoalbidus* (presently ongoing; Katharina Kräutler, doctoral thesis).
- Fungal isolations and wound inoculation experiments showed that leaf symptoms of *F. excelsior* are caused by *H. pseudoalbidus* and that leaves of *F. angustifolia* and *F. ornus* are also susceptible to the ash dieback pathogen (Katharina Kräutler, doctoral thesis; see accompanying poster presentation by Kräutler & Kirisits).
- *Hymenoscyphus albidus* has been rarely collected in Austria and it has not been found in recent years. A few herbarium specimens from Austria, collected prior to the emergence of ash dieback, are presently being examined, in order to unambiguously confirm their identity as *H. albidus* (Katharina Kräutler, doctoral thesis).

- From 2009-2012, the intensity of dieback and leaf symptoms was investigated on *F. excelsior* clones in three Austrian seed plantations. In agreement with studies in other European countries, great variation in the damage levels of different clones was detected, indicating that they differ substantially in their resistance to the ash dieback pathogen (Christian Freinschlag, Master thesis).
- In order to investigate the disease cycle of ash dieback, a symptom observation study on ash seedlings has been initiated in two forest nurseries in March 2011 (still ongoing in one of the nurseries). At monthly intervals seedlings are inspected for the occurrence of ash dieback symptoms. The phenology of sporulation of *H. pseudoalbidus* is investigated with spore traps and by observing ascocarps (Peter Kritsch, doctoral thesis).
- As ash dieback is of high practical importance, considerable efforts were made to disseminate new research findings and recommendations to forest authorities, forest owners and other practitioners. Activities included articles in journals for practitioners, the production of leaflets and lectures at seminars and national meetings.

The Austrian team attending the meeting in Vilnius also consists of tree geneticists, which have been involved in some ash research:

- Berthold Heinze and co-workers conducted a population study of *F. excelsior*, analyzing six seed lots and two seed orchards in Austria at six microsatellite loci (see the accompanying poster presentation by Fussi at al.).
- Heino Konrad is involved in the programs of seed orchards of forest trees and gene reserve forests in Austria. The latter include a number of stands with *Fraxinus* spp.
- Marcella van Loo is presently doing a project on the species diversity and genetic diversity of managed and unmanaged poplar forests; at her study sites she recorded characteristics of forests stands containing also *Fraxinus* spp.

Research needs regarding ash dieback are immense and we are confident that the FRAXBACK COST Action will strengthen both national and international collaboration.

This country presentation is accompanied by the following six posters presented by Austrian participants (presenting authors are underlined):

- Fussi, B., Koziel, A., Lexer, C., <u>Heinze, B.</u>: Is identification of ash seed material possible? (see abstract below)
- Keßler, M., <u>Cech, T. L.</u>, Brandstetter, M., Kirisits, T.: Dieback of ash (*Fraxinus excelsior* and *Fraxinus angustifolia*) in Eastern Austria: Disease development on monitoring plots from 2007 to 2010.
- <u>Kräutler, K.</u>, Kirisits, T.: The ash dieback pathogen *Hymenoscyphus pseudoalbidus* is associated with leaf symptoms on ash species (*Fraxinus* spp.).
- <u>Kirisits, T.</u>, Cech, T. L.: Alien forest pathogens in Austria – a synthesis.
- Kirisits, T., <u>Kritsch, P.</u>, Kräutler, K., Matlakova, M., Halmschlager, E.: Ash dieback in forest nurseries in Austria.
- <u>Van Loo, M.</u>, Schratt-Ehrendorfer, L.: Natural succession of softwood riparian forests: occurrence and distribution of *Fraxinus* spp. (see abstract below)

Table 1: First records of ash dieback symptoms and of *Hymenoscyphus pseudoalbidus* (*Chalara fraxinea*) isolated from symptomatic ash trees in each of the nine Austrian provinces from June 2007 to June 2011. For the province of Tyrol data are shown separately for Eastern and Northern Tyrol.

Austrian province	First record of ash dieback symptoms	First record of *H. pseudoalbidus* (*Chalara fraxinea*)		Recorded *Fraxinus* hosts	Number of localities[3]
		Month & year	Locality, political district		
Burgenland	2008[1]	May 2010	Breitenbrunn, Eisenstadt-Umgebung	*F. excelsior*	4
Carinthia	2008[1]	August 2008	Saberda, Klagenfurt-Land	*F. excelsior*	16
Lower Austria	2005[1]	August 2007	Langau, Scheibbs	*F. excelsior* *F. angustifolia* subsp. *danubialis* *F. pennsylvanica*	41
Salzburg	2005[1]	October 2008	Fuschl, Salzburg-Umgebung	*F. excelsior* *F. excelsior* ‘Pendula’	14
Styria	2006[1]	June 2007	Altaussee, Liezen	*F. excelsior* *F. excelsior* ‘Pendula’	17
Eastern Tyrol	2010[1]	July 2010	Dölsach, Lienz	*F. excelsior*	2
Northern Tyrol	2008[2]	October 2009	Brixlegg, Kufstein	*F. excelsior*	11
Upper Austria	2005[1]	June 2007	Edt bei Lambach, Wels-Land	*F. excelsior*	13
Vienna	2007[1]	January 2008	Neuwaldegg, Hernals	*F. excelsior* *F. excelsior* ‘Pendula’	15
Vorarlberg	2008[2]	May 2009	Götzis, Feldkirch	*F. excelsior*	8
Total		-	-	-	141

[1]Unambiguous observations by T. L. Cech or T. Kirisits. The records do not necessarily reflect an exact time sequence of the occurrence of ash dieback in the various provinces, as they were made during opportunistic and not systematic surveys. It is, however, safe to conclude that ash dieback occurred later in western, southern and south-western Austria (provinces of Carinthia, Tyrol and Vorarlberg) than in other parts of the country.

[2]Reports by staff of the Provincial Forest Authorities of Tyrol and Vorarlberg.

[3]Number of localities where *H. pseudoalbidus* (*C. fraxinea*) has been isolated from diseased ash trees. The differences in the number of records of the ash dieback pathogen in the various provinces do not allow inferring about the intensity of ash dieback in various parts of Austria but just reflect differences in the intensity of sampling efforts.

AUSTRIA: Is identification of ash seed material possible? (POSTER)

Barbara Fussi[1], Agnieska Koziel, Christian Lexer[2], Berthold Heinze

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1 present address: Bavarian Office for Forest Seeding and Planting (ASP), Forstamtsplatz 1, 83317 Teisendorf, Germany
2 University of Fribourg, Department of Biology, Unit of Ecology & Evolution, Chemin du Musée 10, CH-1700 Fribourg, Switzerland

If ash (*Fraxinus* excelsior) will survive the current epidemic, it will subsequently be necessary to restore the tree populations to pre-disease levels of genetic variation. We used microsatellite markers to study the genetic structure of common ash in Austria, in order to give recommendations for seed harvest and establishment of seed orchards, in a project that started right before the symptoms of the disease became wide-spread. Six seed lots (572 individuals) from across the range of the species in Austria, and two seed orchards (130 individual clones) located in northern and southern Austria were analysed at six microsatellite loci.
We discovered high genetic diversity in seed stands and seed orchards, though alleles in the seed orchards were often found in a homozygous state. Whereas a high proportion of diversity derived from pollen in the seed stands, this is undesirable in seed orchards, where reproduction is wanted only among selected trees isolated from the surrounding area. In that case, genetic diversity among the seed orchard clones has to be sufficiently high, because no diversity from outside would be added to the gene pool.
We estimated a generally low level of genetic variation, measured with these markers, among the seed stands (F_{ST}=0.05). Instead, there was relatively high variation among half-sib families (F_{ES}=0.10-0.15). Therefore we suggested to harvest at least from a minimum of 10 to 15 trees per seed stand, and to select different trees and stands each year, for commercial harvests. When seed is harvested repeatedly from the same stand, it should be possible to confirm this with genetic data (identification and traceability). Moreover, from judging the allele size ranges, we discovered that similarities between Austrian and Bulgarian (literature-derived) data are greater than those with French data. This should inform possible seed transfer across Europe. Surprisingly, the genetic composition of our investigated seed orchards did not fully represent the seed stands of similar geographic region. Hence we recommended to examine genetic structure of seed stands and seed orchards in more detail and to genetically investigate material prior to the establishment of seed orchards. In light of the present die-back within the seed orchards, it will be imperative to figure out a way how to restore pre-disease levels of genetic variation for a future restoration attempt.

Keywords: *Fraxinus excelsior*, seed orchards, seed stands, microsatellites, genetic diversity

AUSTRIA: Natural succession of softwood riparian forests: occurrence and distribution of *Fraxinus* spp. (POSTER)

Marcela van Loo[1] and Luise Schratt-Ehrendorfer[2]

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The size and composition of riparian forests located along the River Danube have considerably changed in the last 200 years. This is largely related to *human activities* such as river canalisation, the construction of flood protection dikes and hydropower stations, plantation of non-native species, and forest management. In addition to this, the existing fragments of the former riparian forests are also exposed to *fungi activities* such as Dutch elm disease, and ash dieback.
Fraxinus excelsior and *Fraxinus angustifolia* are tree species of harwood riparian forests which in natural succession replace species of the softwood riparian forests (*Populus* sp. and *Salix* sp.) Using plant-sociological analyses in five softwood forest stands and detailed tree- and shrub-survey on 50m x 50m plots, we studied the natural succession of these stands with respect to the occurrence and the distribution of *Fraxinus* sp. compared to other native hard-wood-, as well as non-native species.

key words: *Fraxinus excelsior, Fraxinus angustifolia*, species composition, stand structure, natural succesion

Situation with ash in Belgium – collaborative work

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Ash (*Fraxinus excelsior*) is present everywhere in Belgium, as a forest tree but also as a roadside tree or in hedges, together with other woody species. In forests, it is found mostly in Wallonia where it is the third broadleaf species after oak and beech. It is present in 37000 ha of forest stands located in two main areas, in the centre and the Southern part of Wallonia, respectively. Ash trees are often present in mixed forests with other hardwood species. Pure stands of ash trees represent only 7700 ha and are mostly in private ownership. A phytosanitary survey conducted in 2004 and 2005 in Wallonia showed that their health status was good[1]. Between 2009 and 2011, a network of monitoring plots was established in Wallonia in order to evaluate the importance of ash dieback caused by the fungus *Hymenoscyphus pseudoalbidus*. Samples were collected and analysed using a real time PCR test developed to detect the pathogen in different plant tissues (leaves, petioles, wood, twigs)[2]. This method is very specific

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(no detection of the closely related species *H. albidus*) and provides results within one working day. Ash dieback was first reported in 2010 on young naturally regenerated trees[3]. In 2011, the disease was found in 66% of the survey plots while only 6% of the plots were infected in 2010. In addition, the pathogen was identified in forest nurseries. The number of disease reports in stands still increased in 2012 through monitoring carried out by members of the Walloon Forest Health Service who were trained in the identification of symptomatic trees. A similar evolution was observed in Flanders[4], and the disease is to date widespread throughout the country.
Plots with ash dieback were selected in Wallonia in pure and mixed stands of mature, adult and juvenile trees in order to monitor the evolution of the disease over several years. First observations were made in 2012 by determining a disease index based on various parameters such as defoliation, presence of dead branches and wilting in the crown, presence of trunk cankers and collar necrosis. In two of the three most affected plots, two trees died in 2012 (among a total of 250 trees) and collar necroses were observed in four trees. Trunk cankers were relatively rare while the level of defoliation was highly variable between stands. Important variation in the level of defoliation was also observed among trees of the same stand.
A procedure combining the use of electric aerial spore trapping and pathogen detection by real time PCR was developed to determine the periods of ascospores release throughout the year, and to evaluate the maximum distance travelled by an ascospore from an infected site. Analyses conducted in 2011 showed that the method was effective to detect the pathogen in the air. Ascospores release was observed from July to October, with a higher production in the beginning of July. The study will continue in 2012 and 2013.
A collection of *H. pseudoalbidus* isolates collected in Wallonia between 2010 and 2012 was established. These strains will be used to assess the genetic and phenotypic diversity of the pathogen at the regional level.

Situation with *Fraxinus* spp. in Bulgaria: stand characteristics, state of health, ongoing research and research needs

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The total afforested area amounts to 4.114 M ha, with broadleaved and coniferous forests occupying respectively 69.8 % and 30.2 % (1). Three *Fraxinus* species are naturally distributed in the country (*Fr. excelsior* L., *Fr. oxycarpa* Willd. and *Fr. ornus* L.), but two other ash species (*Fr. americana* L. and *Fr. pennsylvanica* Marsh.) are successfully introduced as well. The high-stem forest stands of the two most economically important ash species, common ash (*Fr. excelsior* L.) and narrow-leafed ash (*Fr. oxycarpa* Willd.), cover a common area of approximately 15 000 ha with a total timber volume being figured out at above 2.5 M m^3. The last two species have a share of 1.7 % and 1.4 % of the total area and total timber volume of the high-stem broadleaved forests, respectively. As regards the covered area the flowering ash (*Fr. ornus* L.) dominates amongst the young (1-10 years) *Fraxinus* spp. stands (230.9 ha) followed by the narrow-leafed ash (*Fr. oxycarpa* Willd.) (167.7 ha) and the common ash (*Fr. excelsior* L.) (109.3 ha). There are 20 registered *Fr. oxycarpa* (231.1 ha) and 41 *Fr. excelsior* (63.9 ha) seed-producing stands in the country. A study on assessment of the genetic structure of local

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common ash populations revealed significant spatial genetic structure within 4 out of 10 populations as well as among populations, with differentiation among populations explaining only about 8.7% of the total genetic diversity (2).
In a study on the health status of *Fr. excelsior* and *Fr. oxycarpa* stands and plantations realized during 1986-90 in North-Eastern Bulgaria significant withering of trees has been found ranging within 10-49 %. Necrotic lesions and cankers were observed on the braches and stems of the infected trees, with damages being associated with *Cytophoma pulchella* (Sacc.) Gutner, *Endoxylina astroideae* Rom., *Cytospora* spp. Ehrenb. and *Phomopsis* spp. (3). In 2010 damages caused by the fungus *Hysterographium fraxini* (Pers.) de Not were established on 55-year-old *Fraxinus excelsior* L. trees in the same region. Bark necrosis of stems and branches, growth failure, drying and death were observed with 93 % of trees (Rosnev, Petkov, unpublished).
As a party to the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP) since 1985, Bulgaria is a partner country in the International Cooperative Programme on the Assessment and Monitoring of Air Pollution (ICP Forest). As such, the country undertook definite engagements by participation in both Monitoring Level I & II activities, thus annually surveying a national network of 159 sample plots.
Currently, *Chalara fraxinea* is not on the quarantine list of the national phytho-sanitary control authorities. Nevertheless, the increasing threat of the harmful pathogen causing ash dieback across Europe deserves an immediate attention. An implementation of thorough survey on the risky ways for the possible penetration and spreading out of the pathogen should be prioritized. Dissemination of basic knowledge about the specific features of the fungus to a broad auditorium of parties concerned (sylviculturists, nurserymen, forest owners, breeders, etc.) combined with application of modern biotechnological methods for identification and studying the host-pathogen interactions might be the tools of crucial importance for the successful control over the devastating fungus.

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Situation with Ash in the Czech Republic

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The ash dieback was firstly observed in the half of nineties in the Czech Republic. The massive development of the disease started in 2003 and the presence of the pathogen was firstly confirmed in 2007 in southern Moravia. The first wave of the disease roughly moved from NE to SW through the area of the Czech Republic. The pathogen is distributed in the whole area at present. The pathogen is known from both ash species native in the area – *Fraxinus excelsior* a *F. angustifolia.*

Ash is cultivated in ca 36 000 ha of forests, which is 1,4% of the whole forest area in the Czech Republic, especially in humid stands in ash-alder mixed forests and in hardwood forests of lowland rivers. These stands are highly affected by the disease at present. The ash in ravine forests and slopes are usually affected in less extent. The pathogen has also caused important losses in nursery production, new ash forest plantings and young plantations. Ash dieback poses an important risk for riverbank stands because it is an important component (1/3 of trees in some river basins) of such stands in different elevations. Moreover, the co-occurrence of *C. fraxinea* and *Phytophthora alni* together with *Ophiostoma novo-ulmi* in ash-alder mixed riverbank stands poses a serious problem for management of these areas. The high impact of *C. fraxinea* is evident also in landscape plantations, alleys, urban greenery, etc. of various ages.

There is one basic research project funded by Ministry of Agriculture (no. QJ1220218, 2012 – 2016) focused on ash dieback in the Czech Republic.
The sub-goals of the project are:
1) To determine the spread and the importance of *C. fraxinea* in the Czech Republic.
2) To identify the population structure of *C. fraxinea* in the Czech Republic.
3) To determine *C. fraxinea* susceptibility to fungicides.
4) To determine the composition of endofytic mycoflora in ash twigs.
5) To select the endofytic isolates with antagonistic activity against *C. fraxinea*.
6) To study the pathogen biology with emphasis on development of effective protective measures.
7) To identify and preserve *F. excelsior* genotypes with significant level of resistance to *C. fraxinea.*
8) To develope a methodology of cultivation of ash trees in environment with presence of *C. fraxinea*.

The following institutions participate in this project:
- Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Průhonice, Czech Republic
- Mendel University of Agriculture and Forestry in Brno, Czech Republic
- Charles University in Prague, Czech Republic
- Forestry and Game Management Research Institute, Strnady, Czech Republic
- The Forest Nursery Association of the Czech Republic
- Elbe River Basin, State Enterprise, Czech Republic

Three other minor projects (funded by Ministry of Education and Ministry of Agriculture) or their parts are also focused on ash dieback. The aim of these projects is to study of the dependence of disease impact on environmental factors including type of vegetation cover, altitude, exposition, air humidity, temperature, etc.

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Situation with Ash in CROATIA: stand characteristics, health condition, ongoing work and research needs

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Chalara fraxinea T. Kowalski is a novel disease responsible for common ash (*Fraxinus excelsior* L.) dieback revealed during last 10 years in many European countries. The disease was also obtained on narrow-leafed ash (*F. angustifolia* Vahl.), and on American and Asian ash species. The presence of disease on common ash in Croatia was first recorded in Gorski Kotar region in 2009, affecting trees of different ages.

The symptoms of dieback observed in tree crowns were wilting and premature leaf shedding, necrosis of bark and wood discoloration. Pathogen was successfully isolated from necrotic bark and wood tissues collected on symptomatic trees revealed in 2009. In 2010 and 2011, research was expanded on larger territory of Croatia in order to determine distribution of the pathogen. The shoots and branches with observed symptoms were collected from common and narrow-leafed ash. The up-to-day results revealed the presence of *C. fraxinea* in northern and western parts of Croatia, causing dieback in natural stands of common ash in hilly regions and narrow-leafed ash in lowlands.

In May 2011, four permanent sample plots of *F. excelsior* were established in Gorski Kotar region, covering various altitudes from 400 to 1192 m, and three typical phytocoenoses for the area: *Omphalodo–Fagetum*, *Blechno–Abietetum galietosum rotundifoliae* and *Homogino sylvestris–Fagetum sylvaticae*. On each plot, 20 ash trees affected in various stages by *C. fraxinea* were selected for monitoring. The assessment of crown condition (foliage transparency) was done monthly on selected trees from May to October. The results revealed following foliage transparency in 2012 per each site: Luke (altitude 400 m) 5-100 % with average tree transparency 27.8 %; Belevine (altitudes 785-850 m) 5-90 % with average tree transparency 27.3 %; Malo Duboko (altitudes 850-970 m) 0-60 % with average tree transparency 15.3 %; Javorje (altitudes 1130-1192 m) 5-50 % with average tree transparency 13.3 %. According to these results it can be concluded that with higher altitude the foliage transparency decreases, as well as the disease impact to trees in natural forest stands.

Other biotic factors (wood decay fungi, pest insects) and abiotic (frost, windbreak, snow damage) were also recorded as possible additional factors in ash dieback. From April till October ash leaves were collected in litter to find out the presence and development of *H. pseudoalbidus* apothecia.

Isolation of *C. fraxinea* from infected bark and wood on malt extract agar (MEA 2 %, 20g/l Biolife) resulted in growth of morphologically species-specific colonies in diameter of approx. 2 cm after 3 weeks at 22 °C. Kept at 4 °C the cultures obtained phyalides and phyaloconidia after 12 days. Apothecia of *Hymenoscyphus pseudoalbidus* Queloz, Grünig, Berndt, T. Kowalski, T.N. Sieber & Holdenr. were developing on leaf petioles from previous year in the litter throughout Summer and early Autumn.

In 2005 the first *F. angustifolia* clonal seed orchard was established in the seed region "Middle Sava River", on area of 3.5 ha, containing number of 56 clones, spacing 4×4 m. The second clonal seed orchard was established in the seed region "Upper Sava River", on area of 7.3 ha, containing number of 50 clones, spacing 5×5 m. Up-to-day the symptoms of disease have not been found and the presence of *C. fraxinea* have not been confirmed.

Research needs
Molecular analyses of hosts (healthy and symptomatic ash trees) from the research sites and *C. fraxinea* (*H. pseudoalbidus*) from plant tissues or pure cultures.

STSM interest
Lea BARIĆ, MC Substitute Member at COST Action FP1103, is working on ash dieback in Croatia and *C. fraxinea* is her PhD thesis subject. According to which she is interested in STSM in Austria, at University of Natural Resources and Life Sciences (BOKU), Vienna and Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW), Vienna.

Ash in Denmark anno 2012

[1]Lene R Nielsen (presenting), [1]Lea V McKinney, [1]Iben M Thomsen, [2]Ditte C Olrik, [1]Erik D Kjær
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Background and health status
Common ash (*Fraxinus excelsior*) is an important tree species in Denmark, until recently occupying 3-4 % of the forest area. The first symptoms of ash dieback were seen in 2002-03, however not acknowledged as such until 2005. Based on data from the national forest health monitoring it is evident that the entire Danish population of ash is affected. While the disease at the beginning primarily damaged younger trees, systematic health score surveys of ash stands of different ages (9 - >100 years old) have revealed that also older trees are now badly affected. Observations on diameter growth through time show a reduction in growth after the arrival of the disease.

Material
Clonal seed orchards and offspring trials of Danish origin established by the Danish Nature Agency from the late 1990'ties and early 2000's respectively, have formed a unique basis for systematic studies of health, phenology and inoculation experiments. The clonal seed orchards consist of 39 repeated grafts grown at two sites, Tuse Næs (Eastern Denmark) and Tapsøre (South-Western Denmark). F1 and F2 offspring from a sub-set of the clones are also grown in field trials. Also, offspring groups from 101 mother trees are grown at two sites, Randers and Silkeborg, both in Western Denmark.

On-going research projects
Our research mainly focuses on the host. We have revealed large and significant variation in susceptibility among the tested 39 clones from Tuse Næs and Tapsøre during the assessment period (years 2007, 2008, 2009). The genotypic correlation between the two sites was very high during the three years (r_G = 0.9, 0.95, 0.96) indicating negligible genotype-by-environment interaction. Based on the offspring trials from Randers and Silkeborg we find high levels of additive variation in the degree of susceptibility with relatively low influence of environmental factors (narrow sense heritability = 0.37-0.52). Thus, being non-susceptible seems to be inherited from parent to offspring. Controlled inoculation experiments have further shown that clones that are less susceptible to the disease also have slower necrosis development after controlled inoculations with the fungus presumably due to an active resistance mechanism.

The occurence of disease symptoms in Denmark seems to coincide with presence of fruiting bodies of *H. pseudoalbidus* type. While herbarium material prior to 2005 were of *H. albidus* type, herbarium samples from 2005 (collected as *H. albidus*) and new 2010 collections were all *H. pseudoalbidus* indicating extinction of *H. albidus* at least locally in the country.

Current research

Our current research focuses on 1) Transcriptome studies 2) Susceptibility in other species of ash 3) Phenology in relation to the disease 4) Inbreeding depression in relation to the disease. The transcriptome studies compare gene expression profiles of resistant and susceptible clones of *F. excelsior*. In the long run we hope to be able to identify candidate genes involved in the resistance mechanism in ash.

Due to the collection of ash species (around 22 species) at the arboretum in Hørsholm, North Eastern Denmark, we are able to explore the level of natural infections, screen symptomatic tissues for the fungus with DNA markers, assess necrosis development after controlled inoculations, and detect fruiting bodies on decomposing leaves. These results will tell us which other species are susceptible to the disease – and which are not. We may get closer to the origin of the fungus.

We find a significant correlation between leaf coloring and health score. Healthy trees in terms of ash dieback yellow earlier than unhealthy trees. We are currently exploring this relationship by detailed phenology studies in both clones and offspring groups.

Another research project concentrates on inbreeding depression and disease tolerance. This data set is based on F1 and F2 offspring from a subset of the 39 tested clones. The results will perhaps reveal the future scenario of ash facing low populations numbers and increased inbreeding after the bottlenecks caused by the disease.

Other on-going projects

We believe that only very few percentages of the Danish ash gene pool are resistant clones. In order to ensure the future population of ash – of reasonable population size – the University of Copenhagen and the Danish Nature Agency have initiated a programme for the mobilization of healthy clones and later on the establishment of future seed orchards. So far we have identified more than 100 trees in private and state-owned forest. These trees will be revisited during winter for scion collections. We plan to inoculate the grafts in order to select only the best clones. In line with this programme we have performed controlled crossings between the best clones at Tuse Næs (mothers) and the best fathers from Tuse Næs, Randers and Southern Sweden (Swedish pollen from the tested clones at Ekebo).

Research needs

Many things are still unexplored. But we believe that more studies are needed on the interaction between the host and its pathogen in order to understand the biology of the fungus. Simple questions as where in the tree do we find the fungus are still unanswered. We also believe that effort should be put into establishing a controlled method for inoculations with spores rather than wood plugs with mycelium.

***Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*) in Estonia**

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In Estonia the forest land area, where Common ash (*Fraxinus excelsior*) is the dominant tree species reaches only 0.8% and growing stock value by this dominant only 0.7% of the total forest land. It means that ash is not an economically important tree species in Estonia. However, Common ash has high biological value.

In North-West Estonia the first record of *Chalara*-symptomatic Common ashes was detected in 2003, thereafter 2005 in Central and 2006 in South-Estonia. Some uncontrolled information came from foresters about symptomatic ashes, which were noticed already from the middle of 1990s. It seemed that in Estonia the ash dieback has spread from North-West to South-East and not an any opposite way as it has happened with other current invasive pathogens (Drenkhan and Hanso 2009).

The population study of *C. fraxinea* by using 32 samples of Estonia, Finland and Latvia. In this study, RAMS fingerprinting revealed considerable genetic variation among the isolates and high number of haplotypes (14 among 32 isolates). This kind of variation should be typical to native fungal populations and not for new species (Rytkönen *et al*. 2011). However, the first specimens of *Hymenoscyphus albidus* from Estonia were deposited in mycological herbariums only in 2000, i.e. no any earlier findings of the fungus from the mycologically comparatively well-investigated Estonia. Using the species-specific markers (Husson *et al*. 2011) we did not detect *H. albidus* from ash petioles, collected on 5 occasional symptomatic ash stands in 2010. Only *H. pseudoalbidus* was found on the collected ash petioles.

During the autumn 2009, several introduced as ornamentals exotic ash species (*Fraxinus nigra*, *F. pennsylvanica*, *F. americana* and *F. mandschurica*), showing ash dieback symptoms were investigated. All these turned to be infected by *Chalara fraxinea. F. nigra* trees were badly affected, *F. pennsylvanica* trees were moderately affected, *F. americana* and *F. mandschurica* were least affected. In 2012 the infection level of these exotic ashes was evaluated as quite similar, except of *F. pennsylvanica*, which was somewhat less affected than 3 years ago.

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Fraxinus **and** ***Hymenoscyphus pseudoalbidus*** **in Finland**

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Common ash (*Fraxinus excelsior*) is the only indigenous ash species in Finland, and natural ash forests are found only in the most southern parts of the country. Common ash is cultivated mostly as an amenity tree along the native range in the south, but also in central parts of the country in areas with suitable soil properties. Red ash (*F. pennsylvanica*) has adequate frost tolerance for Finnish conditions, and it is also planted in parks and gardens. Other ash species are used rarely. At the moment, no commercial ash seed is produced in Finland. Two officially approved seed collection sites have existed, but have not been used since 1999. Also the only official ash seed orchard has been withdrawn from production in 2005.

Since the beginning of the 2000s, symptomatic ash trees have been observed in the Åland. On the mainland, the first clear signs of disease were observed in 2007. Common ash stands of many ages (from 5 to 100) were sampled in Åland and mainland Finland in 2010 and the ash

dieback fungus (*Chalara fraxinea*, teleomorph *Hymenoscyphus pseudoalbidus*) was isolated for the first time (1). The sampled trees were growing as roadside trees, in parks or in forest stands. One to four symptomatic (i.e. having necrotic lesions) twigs or branches were collected from all the sites. The resulting twenty Finnish isolates as well as one Latvian and 11 Estonian isolates were analysed by random amplified microsatellite (RAMS) markers. The analysis of 14 RAMS markers revealed 6 variable and 8 non-variable ones, and considerable amount of genetic variation was detected among the markers (the 31 isolates were grouped in 17 haplotypes). Analysis of molecular variance among three populations (Åland, mainland Finland and Estonia) found no genetic differentation between populations.
Leaf petioles of ash were collected in the fall 2011 from the groud in city parks in three locations in southeastern Finland. After incubation of two months *H. pseudoalbidus* fruiting bodies were found and the fungus was possible to isolate, confirming the presence of the disease also in the eastern parts of the Finnish coast. So today, the disease is present everywhere the host is present in Finland. In four sites, ash trees have been monitored by photographing since 2008, and the condition of single trees seems to vary from year to year.
In the fall 2011, seeds were collected from both symptomatic and seemingly healthy ash trees (all together 25 trees) in five locations (forest and amenity situations). The seeds were sown in 2011, and the 1862 seedlings will be planted in the autumn 2014 with the aim to screen for possible resistance among Finnish ash genotypes against natural and artificial inoculum of *H. pseudoalbidus.*

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Situation with Ash in France: stand characteristics, health condition, ongoing work and research needs

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In France, research on ash dieback has begun in 2008 further to the first report of *Hymenoscyphus pseudoalbidus* in northeastern France. Then, the disease has quickly spread throughout the north of the country and has reached the north-west coast (Normandy) in 2012. The disease is now well established as severe dieback of *Fraxinus excelsior* and mortality has been observed in the north-east where the pathogen was firstly detected. We worked on the epidemiology of the disease, in particular on pathogenicity and genetic characterization in order to show that *H. pseudoalbidus* is an invasive pathogen. Our recent researches are focused on environmental factors affecting disease prevalence, impact of the pathogen on host population demography and dispersal ability using real-time PCR assays.
From a breeding point of view, INRA aims at exploring the potential for resistance/tolerance in a network of 17 field trials that were installed between 1985 and 2009. These trials contain *F. excelsior* provenances from throughout Europe. Most of the material is organised as half-sib

progenies thus allowing to access additive genetic variance estimates. A privately owned *F. excelsior* seed orchard located outside the chalara infested zone will also undergo laboratory assessment for resistance.

Ash dieback: situation and research in Germany

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Common ash covers some 214.000 ha, i.e. 2.0%, of the forest area in Germany. The highest regional proportion of about 4 % is found in Baden-Wuerttemberg and Mecklenburg-Vorpommern. In some regions, huge areas have been planted with ash in the last two decades, both for ecological reasons and to decrease the risk of climate change. Ash dieback was first observed in Germany in 2002, the first record of *Chalara fraxinea* was made in 2007 in northeastern Germany (Schumacher *et al.* 2007). In southern Germany, ash dieback became evident in 2009, though retrospective analyses of necroses suggest presence of the pathogen since 2006. Annual surveys on kind and severity of tree damage have been being performed since 2009, showing quite a dramatic increase in the number of threatened stands. In the north, salvage cuttings have been necessary since about 2005; in the south noticeable removal of damaged or dead trees has been occurring since 2011. Tree mortality is still rare in the older age classes, and in such cases it is often connected with *Armillaria* sp..
In Bavaria, disease progression and severity has been studied in 24 test areas, taking into account differences between habitats, pure and mixed stands, as well as age. In 2012, in six investigated pole-forest stands, 26% of the trees were dead. In eight mature forests only about 2 % of the trees are dying. The most severe damage was found in ten natural regeneration areas with a mortality rate of about 43%. In four different stands, 600 leaf petioles were examined for the presence of apothecia. The climax of their development was reached at the end of July. Subsequent to sporulation, infection occurred and leaf spots became visible. Intensive fungal growth studies have been started in order to find means to avoid infection or at least to lower the infection pressure by inhibiting either sporulation or growth of the teleomorph.
In a provenance trial established in 2005 in Baden-Wuerttemberg, data on tree growth and symptoms of the disease have been collected annually since 2009 (Metzler *et al*. 2012). The trial area comprises plots on four different sites, and eight provenances of *Fraxinus excelsior* with a total of 1,916 plants. The proportion of affected trees increased from 13% in 2007 to 88% in summer 2011. In 2011, 69% of the trees of the provenance with the significantly lowest disease incidence showed symptoms, contrasting to 95% of the trees of the provenance with the highest incidence. Mortality adds up to 5.6%. Radial increment was diminished by up to 26% (corresponding to a reduction of basal area increment by 45%) in severely damaged ash individuals with bark necrosis as compared to trees without symptoms. Stem quality, as reflected by apical dominance, straightness, epicormic shoots and bifurcation, decreased considerably. The proportion of highest quality stems decreased from 20% to 6%.
Research in Braunschweig centers on studying morphology, physiology, virulence, histology and molecular identities of approximately 200 isolates of *Chalara fraxinea.* These were isolated from diseased *Fraxinus excelsior* in Germany and Poland.
Culture morphology, growth rate and exoenzyme profiles of the isolates varied greatly; there were no correlations between any of these three parameters. Almost all of the isolates secreted tyrosinase and lipase, most of them also secreted β-glucosidase and laccase, some

polyphenoloxidase, peroxidase, cellulase and amylase. Analysis of the ITS1/ITS4 sequence of a selection of these isolates showed that all belong to *Hymenoscyphus pseudoalbidus.* Virulence of the *C. fraxinea* isolates was found to vary greatly following infection of seedlings of *F. excelsior* and *Ocimum basilicum,* a model organism, as well as after application of culture extract in leaf segment tests.
Using dissecting, light, scanning and fluorescence microscopy, apothecial development and structure of *H. pseudoalbidus* were studied, visualizing development and histology of the interaction. The fungus possesses attributes characteristic of most members of the Helotiales: inoperculate asci, development of a primordium, and apparent cleistohymenial development of the apothecia. Haustoria were concentrated in the numerous feeding hyphae beneath the apothecia (de Vries *et al*., in review).
Various approaches are being studied that might be effective in controlling the disease. One of these is to identify a “protective” fungal endophyte. Such isolates from healthy *F. excelsior* are being tested in dual cultures for inhibition of *C. fraxinea.*

Literature:
DE VRIES J; JUNKER C; ROMMEL S; EICKHORST C; SCHULZ B: The apothecium of *Hymenoscyphus pseudoalbidus:* development, structure and control (Forest Pathology, in review)
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Situation with Ash in GREECE: stand characteristics, health condition, ongoing work and research needs

Konstantinos SPANOS, Dionisios GAITANIS, Ioannis MELIADIS, Gavriil SPYROGLOU

Ash species in Greece:
1) FRAXINUS ORNUS (common everywhere in hilly and mountainous areas)
2) FRAXINUS ANGUSTIFOLIA (common in the lowlands – along the rivers and in wetlands)
3) FRAXINUS EXCELSIOR (rare – in the Mountains of N. Greece)

Research Projects – (FP5 – Biodiversity and Ecosystems)
‘Ash species in Europe – Biological characteristics and practical guidelines for sustainable use – FRAXIGEN’

FRAXINUS ANGUSTIFOLIA
F. angustifolia forms even-aged or age-class stands and is mainly found in the forest vegetation zones: QUERCION ILLICIS, OSTRYO CARPINON and QUERCION FRANETTO)
provenance trials – 10 provenance trials (2 at FRI and 8 in central and N. Greece)
silvicultural plots – 2 plots for FAN and 2 for FOR
eventual monitoring/inventory – every year (3 trials) and every 5 years (for the rest).

Silviculture - *F. angustifolia* forms even-aged class-age stands and is mainly found in the forest vegetation zones: QUERCION ILLICIS, OSTRYO CARPINON and QUERCION FRANETTO)

pure or mixed with *Ulmus* spp., *Populus* spp. or *Quercus robur*. It is regenerated by seed (germination in April) and stem and root sprouts (mainly after cutting). It prefers light, humid, rich and deep soils.
Research needs – population studies and gene conservation have priority.

PATHOGENS
Ash dieback – not records (up to now).
Other pathogens – *Phomopsis* spp. (in provenance trials at FRI)
Host – *Fraxinus angustifolia*

INSECTS
Operophtera brumata L. (Lepidoptera – Geometridae)
Attacks various broadleaves and fruit trees. It is a serious defoliator (attacks in April/May) and has been recorded in the last 3 years on *Fraxinus angustifolia*.
Almost all provenances have been damaged. At individual lever, there were found trees (resistant?) not damaged at all or partly damaged (1/3 or ½ of the foliage)
Silviculture – increase planting space?? Phytosanity.
Research needs – look for resistant provenances/genotypes – we have records that genotypes (previously known as *Fraxinus angustifolia* var. *holotricha*) with hairy leaves are resistant to *Operophtera* attack

CANKER
Ash canker - often found on old trees (cause agent: insects or fungi)

FRAXINUS ORNUS
The species *Fraxinus ornus* is mainly found (individually or in small groups) in the forest vegetation zones: QUERCION ILLICIS, OSTRYO CARPINON and QUERCION FRANETTO. It is a light demanded species and has low demands in soil, and can grow on dry, infertile and rocky soils. It usually grows in multi-stems due to the coppicing in the areas were it is found. It is regenerated by seed (germination in April-May), by stem and root sprouts (mainly after cutting or fire).
Health status
In general, no serious diseases or insect attacks have observed apart from some defoliators in very dry periods (summer). Cankers in the main stem and side branches have been observed.

FRAXINUS EXCELSIOR
It is very rare in Greece and found (individually or group of trees) on the high mountains in N. Greece (in the borders with Albania, Fyrom, Bulgaria) in oak, beech or mixed beech-fir forests.
No records (up to now) on ash dieback disease.
Research needs – species inventory, gene conservation and possible ash dieback incidence.

RECENT PUBLICATIONS ON ASH (copies available)
FRAXIGEN. 2005. *Ash species in Europe: biological characteristics and practical guidelines for sustainable use.* Oxford Forestry Institute, University of Oxford, UK, 128 pp. (group of authors).
Verdu, M., Spanos, K.A., Canova, I., Slobodnik, B. and L. Paule, 2007. Similar Gender Dimorphism in the Costs of Reproduction across the Geographic Range of Fraxinus ornus. *Annals of Botany 99*, 183-191.

Papi, R.M., Spanos, K.A., Kyriakidis, D.A., 2012. Genetic variation of *Fraxinus angustifolia* natural populations in Greece based on nuclear and chloroplast microsatellite markers. *European Journal of Forest Research* 131: 1151-1161.

***Chalara fraxinea* incidence in Hungarian Ash (*Fraxinus excelsior*) forests**

The forest area percentages of ashes in Hungary (2006 Forest Inventory data):

Fraxinus excelsior	1,4%
Fraxinus ornus	0,7%
Fraxinus angustifolia	0,4%
Fraxinus pennsylvanica	0,4%
alltogether:	2,9%

Chalara fraxinea was identified for the first time in Hungary in the first half of 2008, in western Hungary near Kapuvár and Sárvár, in 4-6 years old mixed (seed and coppice shoot) origin European Ash (*Fraxinus excelsior*) stands. In the same time in Budapest, under an older Turkey Oak – Sessile Oak – European Ash stand we also detected the symptoms and the pathogen on the saplings of the natural regrowth. With the typical symptoms and the examinations of the collected samples, we were able to definitely identify the *Chalara fraxinea* as pathogen. This pathogenous fungus was also identified on Narrow-leafed Ash (*Fraxinus angustifolia*), from the samples of the Western and Eastern part of Hungary too .

In 2008-2009 we thoroughly researched the distribution of the pathogen in Hungary, and the volume of the caused damages. As a result, we confirmed, that the pathogen spread to the whole area of Hungary. It appears both in young and older stands, but it causes damages more frequently in 2-10 years old forestations. Because of the characteristics of the symptoms and the measures of the dieback, we concluded, that the pathogen appeared in Hungary 2-3 years before. The degree of the infections in the examined forest stands is significantly diverse. The most severe infestation was observed in Eastern-Hungary, near Debrecen, in the summer of 2009. This European Ash stand was 10 years old, with 0,5 ha of area, and was planted with 2 years old saplings. Every single tree showed the symptoms of *Chalara fraxinea* infection.
In the examined part of the forest-stand, the mortality reached 37%. Among the still living trees, the rate of the infected and died stem parts varied between 20-90%. From the symptoms of the dead trees we diagnosed, that the first infections in this area also occurred a few years ago. We do not know much about the environmental conditions assisting the infestation. The examinations of the infested forest-stands of Western-Hungary shows, that the infestation is more frequent on sites with frost-hollow, deep soil and plenty of water. In the same time we also noticed, that the symptoms are also frequent on forest sites drier than average and exposed to extreme cold.. According to the surveys, the fungus is more common in younger stands, but this can be affected by the fact, that we have lesser amount of samples from older and bigger trees, for collecting samples and identifying them from large crowns is more difficult. After the survey in Bükk-mountains, North-eastern-Hungary, we found that the extent of the infection is at least the same on older or middle aged trees, than on the youngest ones. Contrarily, in the western part of the country in mixed species forest stands we experienced mass and severe infections of the natural Ash regrowth, while older trees showed only small degree of typical symptoms in their

crowns. The complete death of older trees takes more time, so major mortality occurs on young, 2-10 years old trees.
In the august of 2009 we surveyed the degree of *Chalara fraxinea* infestations in some forestry's of the Bakony-mountains, in different aged and in different tree-species composition forests. Based on this survey we pointed out, that in the significant majority of the surveyed stands the rate of infected ash trees is under 5%, and in only 2 forest-parts are there 5-10% infestations. Opposite it in 2012 Eastern part of Hungary we found much higher infection of Chalara fraxinea from 22% to 78% in young mixed (Quercus robur and Fraxinus excelsior and Fraxinus angustifolia) plantation and stands.

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

Plans for the future:

- Setting up a monitoring sample-plot network.
  - Data collection on the affected area, affected places, damage intensity, and the temporal changes in damage intensity.
- Selecting resistent ash specimens from natural and cultivated strains.
- Control method examinations, field trials.
- Verification of reproductive materials.

Situation with ash in Ireland: stand characteristics, health condition, ongoing work and research needs

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Fraxinus excelsior is the only ash species indigenous to Ireland. The stocked forest area of ash is 19,200ha (3.1%). Ash is an important species economically, environmentally and culturally and is a significant component in the landscape. It is also a significant component of the Forest Service grant aided planting programme.

The Forest Service, Department of Agriculture, Food and the Marine which is the regulatory authority for forestry in Ireland began surveying for *Chalara fraxinea* in 2008 with surveys intensifying in following years. While ill health has been found in ash, samples consistently tested negative for *C. fraxinea*. There has been considerable trade of ash plants and wood into Ireland in recent years and these have also been monitored.

In October 2012, the first finding of *C. fraxinea* was confirmed at an afforestation site in Co. Leitrim associated with plants which had been imported from continental Europe in 2009. Ash from this consignment had been planted at 11 afforestation sites. The ash at all 11 sites was destroyed by burning. Ireland has now introduced national legislation restricting the movement of ash including plants, seed and wood. The official status of *C. fraxinea* in Ireland is transient: actionable, under eradication.

Before Chalara became a problem, Teagasc has been developing systems to vegetatively propagate mature ash trees using selected plus trees with the view to developing clonal varieties. Material from plus trees have been grafted and developing buds have been established in micropropagation to rejuvenate the material. Thereafter stoolbeds have been produced which give cuttings with a high rooting capacity. This system of vegetative propagation will be discussed; it has potential to build up stocks of genotypes which are confirmed with resistance to Chalara and could be used on material from mature trees and from seedlings.

Situation with Ash in Italy: stand characteristics, health condition, ongoing work and research needs

Nicola Luchi*, Lucio Montecchio**, Alberto Santini*
IPP-CNR, UNIPD***

Stand characteristics
Fraxinus excelsior, *F. angustifolia* and *F. ornus* are widespread in Italy, according to latitude and elevation, in both forest, artificial plantations and gardens. In central Italy *F. ornus* and *F. excelsior* are present in small woods mainly mixed with oaks and pines.

Health conditions
C. fraxinea was detected in 2009 along the Italo-Slovenian border. According to recent surveys, it's widely present in *F. excelsior* and *F. angustifolia* independently from the altitude (0 to 1000 m asl), exposure, soil type, stand age. Never detected in *F. ornus*. It's detected westward ca. 50-60 km /year; nowadays its present above the Po river, in the whole north-eastern Italy. Below this river the fungus has not been detected so far, although declining ashes are present. A recent survey, carried out in Tuscany, showed the presence of ash decline on *F. ornus* and *F. excelsior*, with symptoms resembling those due to *C. fraxinea*. The analysis from this plant material showed the presence of several fungi related with ash decline. *C. fraxinea* was never isolated yet.

Ongoing work and research needs
IPP-CNR (2 post PhD partially involved, 2 researchers partially involved)
1. Monitoring declining ashes in Mediterranean area where *C. fraxinea* wilt is still not reported (Central Italy) (in progress since April 2012).
2. Analysis of endophytic fungal communities involved in ash decline in Mediterranean area (in progress since April 2012).
3. Pathogenicity test of different isolates of *C. fraxinea* (collected from northern to southern Europe) on different Mediterranean provenances of *F. ornus* and *F. excelsior* (Italy and France), in collaboration with INRA (spring 2013).
4. Environmental conditions influencing ash susceptibility and *C. fraxinea* virulence (in collaboration with INRA) (spring 2013).

UNIPD (1 PhD student fully involved, 1 student + 1 technician partially involved)
1. Characterization of both the hyphal ultrastructure and the alterations induced in the woody tissues by means of transmission electron microscopy (on *F. excelsior*; paper submitted).
2. Same on *F. ornus*, of 8 different geographical provenances according to a latitudinal gradient, to verify similarities/differences (since march 2012).
3. Development of new, “ash compatible” injectable fungicides. Laboratory tests ok, artificial inoculations on adult trees successful; by next spring injections of different formulations will be made in both F*. excelsior* and *F. angustifolia*, preventive and curative. In this research 4 units are involved: forest pathologists, chemists, pharmacologists, biologist (since march 2012; € 600.000 grant request submitted).

Common ash (*Fraxinus excelsior*) dieback in Latvia

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Recently described ascomycete *Hymenoscyphus pseudoalbidus* (anamorph: *Chalara fraxinea*) causes severe ash dieback throughout Europe. In Latvia, according to the State Forest Service data (State Forest Service, 2011), area of ash stands (2,189.8 ha, 1% of total forest area) had decreased by 5,628 ha (25.7% of area of all ash stands), in average by 651 ha per year since year 2000. Decrease was more intensive till year 2006 – 843 ha per year, ash stand degradation became slower after 2006 – 235 ha per year. The young ash stands on wet natural sites (on peat and drained peat soils) were more affected.
Fungus *Chalara fraxinea*, the pathogen, which by most authors were admitted as main cause of ash dieback, first time it was found in Latvia by Thomas Kirisits in 2008. In 2010 samples from four sample plots from two different regions of Latvia were sent to Andrin Gross in Institute of Integrative Biology in Switzerland and from approximately 91 sampled twigs 67 *C. fraxinea* isolates were made. In 2011 *Chalara fraxinea* was found also in healthy looking ash seeds.
In order to assess health conditions of ash stands and change of stand composition, 20 permanent sample plots were established in different regions of Latvia during the years 2005-2006. The aim of ongoing research is to clarify changes of silvicultural parameters in declining ash stands.

Situation with Ash in Lithuania: stand characteristics, health condition, ongoing work and research needs

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Stand characteristics. The main current characteristics of Lithuanian common ash (*Fraxinus excelsior*) stands (data from 2011): mean age = 65 years, mean stocking level = 0.5, mean growing stock volume = 208 m^3/ha, mean gross annual increment = 5.7 m^3/ha; mean wood accumulation = -7.2 m^3/ha. Distribution of ash stands by age classes: young, 15.3%; middle-aged, 76.4%; premature, 3.8%, and mature, 4.1%. Due to continuing sanitary fellings, the area of *F. excelsior* stands has decreased from 50.8 thous. ha in 1995 (before the occurrence of dieback) to 36.3 thous. ha in 2011, i.e., from 2.7% to 1.8% of all Lithuanian forest stand area. According to data collected from permanent monitoring plots (State Forest Service), about 10% of ash trees die out every year. There is also a big loss in objects used for conservation of *F. excelsior* genetic resources: due to the ongoing dieback, the one and only ash seed stand and 5 out of 11 former ash genetic reserves are already gone. Today, the density of remaining viable and flowering trees is extremely low (3–5 trees per ha) in most of the remaining (yet, severely damaged) ash stands. Therefore, the effective population size has most likely decreased substantially thus possibly compromising the remaining level of genetic diversity and possibilities for genetically sufficient regeneration of *F. excelsior* populations. As establishment of new ash plantations has been suspended, poor current recruitment rate and health condition of *F. excelsior* self-regeneration is not likely to ensure domination of this species in the future stands: in most cases it is largely outnumbered and suppressed by early-successional tree taxa such as *A. incana, Betula* spp. and *P. tremula* leading to a significant shift in species composition as compared to the situation in pre-dieback stands.
Health condition. The dieback of *F. excelsior* has started around 1995–1996 in forests of North-Central Lithuania. Currently the epidemic is in its chronic phase and virtually all Lithuanian ash stands are damaged to a greater or lesser extent and are subject of the sanitary fellings. In 2011, for example, there were 4.4 thous. ha of heavily damaged ash stands in state-owned forests that were subsequently assigned for sanitary clearfellings; an additional portion comes also from private forests. According to data collected from the permanent monitoring plots (State Forest Service), younger stands (age <50 yrs) are more intensively damaged than the older stands (>50 yrs), however the overall health condition of *F. excelsior* is continuously deteriorating irrespective of stand age. The mean proportion of sound-looking trees in the investigated plots has dropped from 29% in 2008 to 7% in 2012, while the proportion of dead trees has respectively increased from 0% to 45%. In addition to continual infections by *Hymenoscyphus pseudoalbidus*, root rot caused by *Armillaria* spp. is observed virtually in every stand and is seemingly accelerated by *H. pseudoalbidus*-invoked damages on ash crowns. Most of the diseased and dead trees of older age bear symptoms of the advanced root rot.
Artificial re-establishment of ash stands is currently not recommended due to a high risk of infections by *H. pseudoalbidus*, and natural regeneration of *F. excelsior* following clearcuts in the diseased stands is very poor (both in terms of quantity and quality) regardless clearcut age (time since felling), fraction of *F. excelsior* in the former (pre-dieback) stands, site conditions (forest site type) or geographic location.
Ongoing work. Lithuanian researchers are involved into activities of four ongoing research projects, targeted or associated with the ash dieback: 1) an international SNS – Nordic Forest Research Co-operation Committee project "Decline of *Fraxinus excelsior* in northern Europe" (2010-2012; task of the Lithuanian team - investigation of potential of *F. excelsior* self-regeneration following sanitary clearfellings in declining ash stands), 2) recently launched Lithuanian-Swiss collaborative project "Incidence of mycoviruses in epidemic and post-epidemic populations of the ash dieback pathogen *Chalara fraxinea* and evaluation of their potential for biological control of the disease" (2012-2016), 3) a national project "Eco-genetic control of resistance to the pathogen *Chalara fraxinea* in common ash" (2012-2014), and 4) Lithuanian National Programme project „Vulnerability and productivity of local species under biological

invasions and climate change" (a part of the project assigned to analysis of *F. excelsior* stand decline in Lithuania; 2012-2014). Moreover, Lithuania has recently joined the COST programme Action No. FP1103 "FRAXBACK" (2012-2016). Also, a four-year PhD project on ash dieback (studies on epidemiology of *H. pseudoalbidus* aiming to evaluate perspectives of further *F. excelsior* growing and breeding) has been started in 2011.

Completed projects: 1) Lithuanian National Programme project „Vulnerability of native tree species and populations, changes of natural distribution range and forecasts for climate changes" (a part of the project assigned to analysis of overall situation in Lithuanian ash stands and genetic variation of *F. excelsior* in disease resistance; 2010-2011); and 2) a national project „Progeny-based evaluation of *F. excelsior* populations and plus trees for disease resistance, selection of the most resistant genotypes, and preparation of recommendations for establishment of seed orchards" (2010).

Studies on genetic variation in resistance and other adaptive traits of *F. excelsior* are performed in three progeny trials established in 2005 in three different ash provenance regions of Lithuania (Pliūra et al. 2011). In these trials, a total of 140 open-pollinated families from ten Lithuanian populations and 180 open-pollinated families from fourteen foreign European (Belgian, Czech, Danish, French, German, Irish and Polish) populations are being tested. In 2012, a clonal archive and 6 clonal trials-seed orchards have been established aiming to test the performance (growth parameters and resistance to *H. pseudoalbidus*) of progeny-tested most resistant 28 Lithuanian and 22 foreign *F. excelsior* clones and 180 clones selected for resistance in natural populations. Also, in 2012, four progeny trials have been established for testing of 20 families form two Lithuanian ash populations. Further testing of the most resistant clones is carried out trough artificial inoculation with *H. pseudoalbidus*; the new cycle of selection shall provide a possibility of obtaining a set of genetically most resistant clones for crossings in tree breeding and for mass propagation. The relationship between certain phenological traits of *F. excelsior* and its susceptibility to the disease has been investigated in the progeny trials (Pliūra and Baliuckas 2007). Communities of fungi inhabiting *F. excelsior* stems and roots and their impact on health condition of the declining stands have been studied by Lygis et al. (2005) and Bakys et al. (2011). A collection of *H. pseudoalbidus* isolates from all over the country is continuously accumulated and molecular characterization of the pathogen's populations has been started. Small-scale pathogenicity tests have been performed by staff of Dept. of Forest Sanitary Protection at the State Forest Service in 2010-2011 and by Bakys et al. (2011). Ash conservation and breeding programme is coordinated by the Dept. of Forest Genetic Resources at the State Forest Service.

Under the COST programme (Action 'FRAXBACK'), the Nature Research Centre (Vilnius, Lithuania) has hosted STSM by an Ukrainian researcher Dr. Kateryna Davydenko; and two Lithuanian researchers, D. Vaidotas Lygis and PhD student Diana Marčiulynienė are in/plan to go to the STSMs in Latvian and Swedish research institutions.

Research needs. There is a need to study genetic variation in local populations of *H. pseudoalbidus*; to study the rate of latent infections in symptomless ash seedlings in forest nurseries and in natural stands (under forest canopy); to find heritable traits (or genetic markers) of *F. excelsior* associated with its susceptibility to *H. pseudoalbidus* thus enabling straightforward selection of the resistant clones; to investigate disease cycle of ash dieback in Lithuania (concentrating on dispersal patterns of *H. pseudoalbidus* spores throughout the year) and to find its associations to tree phenology.

Ash dieback in the Netherlands

Jitze Kopinga * & Sven de Vries *

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History
It is not precisely known when *Chalara fraxinea* arrived into the Netherlands. Till 2009 there were no dramatic outbreaks of the phenomenon and occasional minor twig dieback was regarded as usual symptoms of the trees response tot sub optimal growing site conditions. In 2010 first reports appeared on cases of dramatic twig dieback of ashes at locations in the north eastern and middle parts of the country. Studies of the plant disease branch of the Netherlands Food and Consumer Product Safety Authority (NVWA) revealed that the causal agent was *Chalara fraxinea*. In 2011 it became also clear that the disease was present in all parts of the country.
In order to get more insight in the phenomenon and its impact on forestry, landscape, urban amenity and tree nursery production, in 2011 a project team was formed within Wageningen University and Research centre i.c. The Centre for Genetic Resources and the Institute for Applied Plant research (PPO) with the following researchers:
ir. Sven de Vries (CGN, Forestry & Forest genetics)
ir, Fons van Kuik (PPO, Control of pests and diseases)
dr. ir. Jelle Hiemstra (PPO, Plant pathology)
ir. Jitze Kopinga (CGN, Tree biology & pathology, project leader)

Funded by the Industrial board for Horticulture (PT) and the State Forestry service (payment in kind) the project group started its activities in the beginning of 2012. PPO aimed its activities on the disease present in tree nurseries and CGN studied differences in tolerance of the host to the disease among a broad variety of clones of ash that were selected during the past decades from about the mid of the last century on and that were conserved in gene banks and seed orchards of the CGN. The same is done for a number of provenance- and progeny trials all over the country.

Stand characteristics
Fraxinus excelsior (common ash) is the only native ash species in The Netherlands and the only species that is used in forestry and the rural landscape. Other -nonnative- species, such as *F. ornus*, *F. americana* and *F. angustifolia* are used as amenity trees in the urban environment only.
In forestry traditionally seedlings of selected progenies and provenances of ash are used as plant reproductive material. Formerly, also in the landscape seedlings were used together with and to a larger extent with clonal selections (cultivars), such as 'Westhof's Glorie', 'Altena' 'Atlas' and 'Eureka'. In the urban environment mainly, if not exclusively cultivars are used.

In the period 2001-2005 (the latest Dutch Forest Inventory) the total forested area in the Netherlands was 359845 ha of which 9800ha (3.1 % of the total forested area) were stands of common ash. Ash in mixed stands represents 5.6 % (16600 ha) of the total forested area. Most ash stands are rather young and were established in the second half of the last century. The average DBH class (stem diameter at breast height) is about 25 cm. Most of the stands are situated in the middle part of the Netherlands at the IJsselmeer polders area (reclaimed land from the former Zuiderzee) and the north western and western part on the peat and clay soils. The same accounts for ash in the rural landscape, although these trees generally are a little bit older.

Health and condition of ash trees
Generally the common as in the Netherlands is not seriously threatened by pests or diseases. Apart from bark canker, caused by *Pseudomonas syringae* ssp. *savastanoi*, other illnesses are of minor practical importance or can be easily controlled by common cultural practices.

During the inventories in 2012 a very broad geographic variability was observed in the intensity of disease attack of *Chalara fraxinea* in forest stands and roadside plantings. As in 2010 and 2011, most of the severe infestations were still recorded in the north eastern and middle part of the country while the south and south west part were virtually still “clean” apart from a few exceptions. Also plantings of seedling ashes in forests or forest like plantings looked much more affected on average than clonal plantings along the rural traffic infrastructure. In contrast with 2011, the disease in all kinds of plantings seemed to be mainly restricted to just leaf infections. The occurrence of bark necrosis and dieback of young shoots was low.
In our clonal trial plots and seed orchards we ascertained a broad variability of leaf infection among clones, ranging from almost 0 to 100 % of the total tree crown volume, indicating that tolerance to the disease might be highly determined by individual genetic factors of the host. Furthermore, it appeared that four of the most widely used amenity cultivars scored “moderate” to highly tolerant for leaf infection, also depending on the age of the tree (young trees appear to be more susceptible). This could partly explain why the disease during the last few years was not so conspicuously present in many of the roadside plantings and in the urban environment.

Ongoing research and research needs
For further breeding and selection of tolerant plant material it is important to know to what extent levels of tolerance of parent trees come to expression in their progenies, and also if certain provenances on average are more tolerant than others.
For this purpose, in 2013 we again plan to investigate the levels of disease attack in our existing progeny and provenance trials.
Since rating of disease attack in these stands is not as easy compared to rating of solitary trees, a quick scan method, if possible by artificial inoculation of trunk tissue could be helpful.
Apart from this, any reliable and quick “objective” method to confirm our findings based on visual classification of the degree of tolerance would be helpful to speed up the process of selection for both forestry purposes (seedlings) as well as cultivars to be used as amenity trees.

Situation with ash and ash dieback in Norway – current situation and ongoing research

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Stand characteristics
Norway hosts the northernmost occurrences of ash in Europe, ash growing in small and fragmented stands along the cost. The tree is conserved mainly in nature reserves, more than 40 nature reserves are registered with ash as one of the dominating tree species. In many

nature reserves however, afforestation, browsing by wild ungulates and competition with the introduced forest tree species *Acer pseudoplatanus* are factors threatening ash stands. Since ash is not an important timber source in Norway, we have no seed orchards, provenance trials or silvicultural plots.

Ongoing work related to genetic characterization of ash

Currently, there is practically no information about the genetic structure of Norwegian ash populations. Characterizing the present genetic resources and comparison with other European populations will provide us with vital information to evaluate conservation efforts, such as management practices and seed sampling strategies. Due to the very fragmented distribution pattern, Norwegian populations may be expected to be genetically more distinct than the more continuous populations further south in Europe. A genetic survey based on microsatellite loci is currently undertaken in Norwegian populations. Ash from 16 nature reserves has been collected and analysed in comparison with samples from other European countries, altogether 1000 samples are being analysed. For all trees collected in Norway, health condition is registered. By characterizing the present genetic structure, we will also have the basic knowledge to gain estimates of the genetic bottleneck the disease may cause in the future.

Danish field trials of ash have shown a genetic component in host resistance towards the ash dieback suggesting variation in resistance also between groups of relatives. Earlier studies from Europe have shown significant clustering of relatives in many ash populations. In two surveillance plots in Norway and in two stands in Lithuania (in cooperation with V. Lygis), we are testing if variation in susceptibility to the ash dieback may be related to certain family groups. In this work, we employ newly developed double digested restriction site associated DNA sequencing (ddRAD tags) to gain a more complete coverage of the genome to facilitate accurate mapping of spatial genetic structure and identification of family groups. The sequence data will also be analysed with regards to susceptibility of ash dieback. In a preliminary test, we are using material obtained from the Danish ash trials (in cooperation with E. Kjærs research group).

Health condition and surveillance plots

We are following the spread of ash dieback in Norway along the west coast, and it has now passed Sognefjorden and more than half of Sogn og Fjordane county is now infected. Only a few trees and a few shoots are infected around the front of the spread.

In south-eastern part of Norway 4 surveillance plots were established in 2009. In 2010 one stand in the southernmost part of the country and in 2012 three plots at the west coast were established. The health condition of the trees in the plots in south-eastern Norway is variable from very little infected to dead or dying. Nearly one third of the small trees are now dead, while the situation for the large trees has remained more or less unchanged since 2009.

Ongoing research related to Hymenoscyphus albidus and H. pseudoalbidus

By employing a real-time PCR assay developed by French colleagues for species-specific detection and quantification of *H. pseudoalbidus* DNA, we have profiled ascospore production of the pathogen over several seasons in a diseased stand in southern Norway. The data show that in Norwegian conditions the infection pressure is maximal in the period between mid-July to mid-August. In collaboration with M.Sc. student Stefanie Steinböck and her supervisor Thomas Kirisits (Austria), we are currently investigating the mode of infection in ash leaves naturally infected by the pathogen. By using ION TORRENT PGM, we have sequenced the genomes of selected Norwegian strains of *H. pseudoalbidus* and *H. albidus* to 20-25X coverage, and are in the process of comparing their genomic similarities and differences. To investigate population dynamics of the pathogen, sequenced rad taq analysis will be carried out for the Norwegian population and European reference strains by the end of 2012/early 2013.

Possibilities for hosting short term scientific missions and next generation sequencing equipment
The Norwegian forest and landscape institute has an active group of researchers engaged in research on *Fraxinus excelsior* and *Hymenoscyphus albidus* and *H. pseudoalbidus*. We would be interested in hosting STSMs interested in cooperation. As a part of our institute's priority program, we recently installed an Ion Torrent PGM DNA sequencer as a versatile and scalable experimental system. This sequencer can produce mean read lengths of greater than 250 bases using semiconductor technology. To enable genome wide profiling analyses, we have developed ddRAD tag protocol that is significantly streamlined and compatible with the Ion Torrent PGM. Furthermore this system is tunable, particularly in relation to the numbers of markers obtained and the number of individuals profiled, allowing us to effectually scale our analysis. Development of a combinatorial barcode system is also underway, and when fully expanded, this will permit the flexibility that several hundred individuals be profiled in a short time.

Polish case study of ash decline in natural reserve Wolica (Chojnów Forest District) and in southern Poland

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The phenomenon of ash decline in Poland is known since 1992. New species *Chalara fraxinea* described by Kowalski (2006) was often present in the upper part of dying ashes and the fungus was found in all age classes. The soil pathogens were neglected for many years due to difficult identification procedure. Nowadays, molecular tools allowed to discover many *Phytophthora* species in the soil rhizosphere of diseased trees. *P. plurivora, P. hungarica*, and *P. megasperma* were found in Chojnów Forest District (close to Warsaw). The two last species were found in Poland for the first time. Their role in fine root damage remain still unknown and need pathogenicity tests as well as the relationships between *Chalara* and *Phytophthora* should be further investigated (B. Orlikowski et al. 2011). From practical point of view it is important to find the way to limit ash decline phenomenon and involved economic problems (ash was excluded as forest tree species in Poland till the situation will stabilize). That is why several trials with phosphates are being performed, now in severely damaged ash stands.
On the basis of 90 RAMS markers *Chalara fraxinea* genetic variability was analysed. Analysis allowed for differentiation of *Chalara* strains into two groups: the first deriving from lowlands and the second from uplands and mountainous areas. Dependency between the elevation above sea level and climatic conditions and intrapopulation genetic variability was confirmed thanks to high statistically significant correlation coefficients. We did not find individuals with identical haplotype. This leads to the conclusion that the only way of *Ch. fraxinea* propagation is creation of ascospores.

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Situation with Ash in Romania: stand characteristics, health condition, ongoing work and research needs

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Stand characteristics

Common ash (*Fraxinus excelsior* L.) is the most important ash tree species in Romania. Along with the other native (*F. ornus* L., *F. pallisae* Wilmott, *F. coriariaefolia* (Scheele) E. Murray, and *F. angustifolia* Vahl.) and North-American ash species (*F. pennsylvanica* Marsh. and *F. americana* L.) it covers about 58,000 ha.

Common ash is found from the plain area to the mid-mountainous zone, up to about 1,400 m elevation asl. As scattered tree, part of upper storey, it is an important component of oak (pedunculate or sessile)-dominated and two-storied stands; sometimes (especially on waterlogged sites, where its quick growth potential is expressed the best) it can form pure stands on small areas.

Health condition

In the state forests, common ash decline has been recorded on 415 ha/year in the last decade (2001-2010), in general (86%) of low intensity. Moderate to high intensity dieback phenomenon had a peak in 2002 (overall area 1,395 ha).

Chronic ash decline was noticed in common ash stands of Dolj County Branch (west of Danube Plain) of State Forest Administration ROMSILVA. In here the dry climate was overlapped to repeated defoliation of *Stereonichus fraxini* (Nețoiu, 2005). Similar phenomenon has occurred in Vaslui County Branch (Moldavian Plateau) of ROMSILVA in the second part of last decade (Blaga, 2010).

Chalara fraxinea (Kowalski, 2007) was not clearly identified in Romania, even if some ash naturally regenerated stands and young plantations of Bucharest Plain (2005-2006) and Prut Meadows (2007-2009) have been affected by rapid dieback. In such stands some disease symptoms (shoot necrotic tissues and dieback) have been noticed (Chira, 2005-2009).

Some other recent mass dying of ash trees have been recorded in plain mixed (pedunculate oak with common ash and other broadleaved species) stands of Siret River (Suceava Plateau, North Moldavia), after the 2010 flooding, having similar symptoms of collar (and root) rot produced by *Phytophthora* sp. in Poland (Popovici and Chira, 2010-2011; Orlikowski, 2011).

Ongoing work and research needs

The Forest Research and Management Institute (ICAS) has a small research project (*Decline of pine, beech, oak, ash etc. – causal factors and stand management*) funded by the State Forest Administration ROMSILVA (research cycle: 2011-2013), with the aim of identification of abiotic and biotic (including the status of *Chalara fraxinea* in Romania) factors involved in ash decline.

Situation with Ash in Russian Federation: stand characteristics, health condition, ongoing work and research needs

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Several species of ash are distributed in the Russian Federation (some sources report 5 in Russia and up to 13 in the former USSR; Buligin, Yarmishko, 2000; Usoltsev, 2001). Among them there are a few native species: *Fraxinus excelsior* (spread widely in Russian), *Fraxinus mandshurica* (in the Russian Far East), *Fraxinus rhynchophylla* (in the Russian Far East, sometimes planted as an urban tree in the European part of Russia), *Fraxinus oxycarpa* (in The Caucases), *Fraxinus coriariaefolia, Fraxinus sieboldiana* (in Russian Far East, very rare), and *Fraxinus ornus.* About 20 species have been introduced to Russia (and in the former USSR). They are widely used in urban greening. The most important of them are: *Fraxinus pennsylvanica*, *Fraxinus lanceolata*, *Fraxinus alba*.
The total area of ash forests (i.e. forests with ash as a dominant tree species) is estimated to be about 760,000 ha in the former USSR, including 250,000 ha in the European part of the country and about 500,000 ha in the Russian Far East. The total timber volume is estimated to be about 120 mln m^3. In some case timber volume can reach 350–480 m^3/ha (Forest Encyclopedia, 1986; Buligin, Yarmishko, 2000; Usoltsev, 2001).
It is generally believed that ash is damaged much less by arthropod pests and pathogens than other tree species and the list of its pests and pathogens is very limited. Our preliminary review of Russian literature shows that it might be not true. The following arthropod taxa are reported to damage different species of ash in Russia:
Arthropoda – 2 classes, 6 orders, 33 families, 95 species.
Insecta – 5 orders, 31 families, 91 species:
Lepidoptera – 15 families, 39 species,
Coleoptera – 7 families, 32 species,
Homoptera – 4 families, 10 species,
Hymenoptera – 3 families, 5 species,
Diptera – 2 families, 5 species,
Acarina – 1 order, 2 families, 4 species:
Trombidiformes – 2 families, 4 species.

Among insect pests there are many monophagous as well as oligophagous species. A few species are able to produce outbreaks and, thus, seriously damage ash: sawflies *Macrophya puctumalbum* and *Tomostethus nigritus*, the Spanish fly *Lytta vesicatoria*, lepidopterans *Abraxas sylvata, Hyphantria cunea, Archips crataegana, xylosteana, Archips rosana, Choristoneura diversana, Operophtera brumata* and *Abraxas sylvata.* At least two species of beetles damage seeds: *Lignyodes suturatus* and *Lignyodes enucleator*. A few species of beetles are considered to be serious pests of ash: bark beetles *Hylesinus fraxini, Hylesinus crenatus* and *Hylesinus toranio*, buprestids *Agrilus viridis* and *Agrilus planipennis*. The Emerald ash borer *A. planipennis* recently became a very serious ash pest problem in Russia and North America. This Asian species was accidentally introduced to North America approximately in 2002 and by now has killed ash trees over a wide area in 13 states of the USA and 2 provinces of Canada. In 2004–2005 this species was recorded in Moscow (Russia) and since then it is actively spreading

around the city. It is highly probable that the species will reach the western border of Russia within a decade (Baranchikov, Kurteev, 2012).
Fungal diseases of ash are poorly studied in Russia. Seeds are known to be damaged by different species of *Heterosporium, Trichothecium* and *Cladosporium* (Deuteromycetes), leaves – by *Uncinula fraxini* (Ascomycetes), *Phyllactinia guttata* (Leotiomycetes), *Phyllosticta fraxini* (Deuteromycetes) and other species. Steams and branches can be damaged by *Cytophoma pulchella* (Deuteromycetes), *Diplodia inquinans, Hysterogrphium fraxini, Massaria vomitora,* (Dothideomycetes), *Endoxylina stellulata* (Basidiomycetes), *Nectria cinnabarina* (Ascomycetes), *Laetiporus sulphureus* (Basidiomycetes), and other species, roots – by *Armillaria mellea* and *Armillaria gallica* (Basidiomycetes).
The emerging new disease caused by *Hymenoscyphus pseudoalbidus* (Ascomycetes) is very new for Russia. Reports suggest that it might have been already found in Kaliningrad region of Russia (Zhigunov et al., 2007). Sampling and subsequent genetic analysis demonstrated that the fungus is present in St. Petersburg too (R. Vasaitis, T. Kirisits, personal communication). Survey recently distributed among phytopathology and forest health specialists in Russia shows that the species has not been recorded and the ash health problem is not yet considered serious in other parts of the country.
There are no doubts that further research on the ash health condition, the pathogen current range, host plant sensitivity, resistance and pathogen control measures are badly and urgently needed.

Situation with Ash in Serbia

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Three *Fraxinus* species are present in Serbian forests. According to the National Forest Inventory *Fraxinus angustifolia* and *Fraxinus excelsion* forests cover 38.000 ha or 1.7% of total forest area and have stock of approximately 3.15 million m^3.
Narrow leaved ash form pure forests along main rivers (Sava, Danube and Morava) or mixed forests with *Quercus robur*. Mixed Pedunculate oak and Narrow leaved ash forest are one of the most productive forests in Serbia. White ash usually forms mixed forests with beach and sycamor. Although, is present in different forest types rarely forms pure forests. White ash and sycamore maple forests occur most frequently at some transitory sites and particularly valuable ecological niches, but also as admixed species with other tree species, especially in belt beech.
Fraxinus ornus form forests with oriental hornbeam and hop hornbeam. These forests grow on extremely unfavorable xerothermic sites, most frequently in gorges, canyons and rocky terrains on about 87,200 *ha*. These forests have low economic value, but they are very important for protection of soil.
Till now no positive isolation of Hymenoscyphus spp. was obtained. Different phases of decline (dead twigs, dieback, defoliation, etc.) are present in all ash forest types, but presence of disease was not confirmed yet. Sampling in different regions with dominance of *Fraxinus excelsior* and *F. angustifolia* is regular.

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Situation with Ash Dieback in Slovakia in 2012

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Forest coverage of Slovakia is 1.9 mil. ha and that is 40 % of the total area of the Slovak republic. The most common tree species is European beech (*Fagus sylvatica,* 31 %), followed by Norway spruce (*Picea abies,* 26 %), oak (*Quercus* spp., 13 %), pine (*Pinus* spp., 7 %), and other tree species (23 %).

Ash (*Fraxinus* spp.) is not one of the main forest tree species in Slovakia, but it is quite common in small landscape forests and parks. It prefers wet, stony soils and is widely distributed throughout Slovakia. Trees form small forest stands, usually up to 1 ha, at the bottom of slopes or by rivers. There are two species: *Fraxinus excelsior* L. and the much less common *Fraxinus ornus* L. They cover 1.5 – 2 % of forest land, equivalent to approximately 30 000 ha. Within the established system of genetic resources to June 1, 2009 records in Slovakia:

- 9 genebase for the area of 1758.78 ha (122.81 ha represented ash – 7 % share),
- 340 approved stands to collect seeds on an area of 2912.74 ha (616.40 ha represented ash – 21 % share),
- 206 plus trees,
- 3 seed orchards on the area of 1.80 ha.

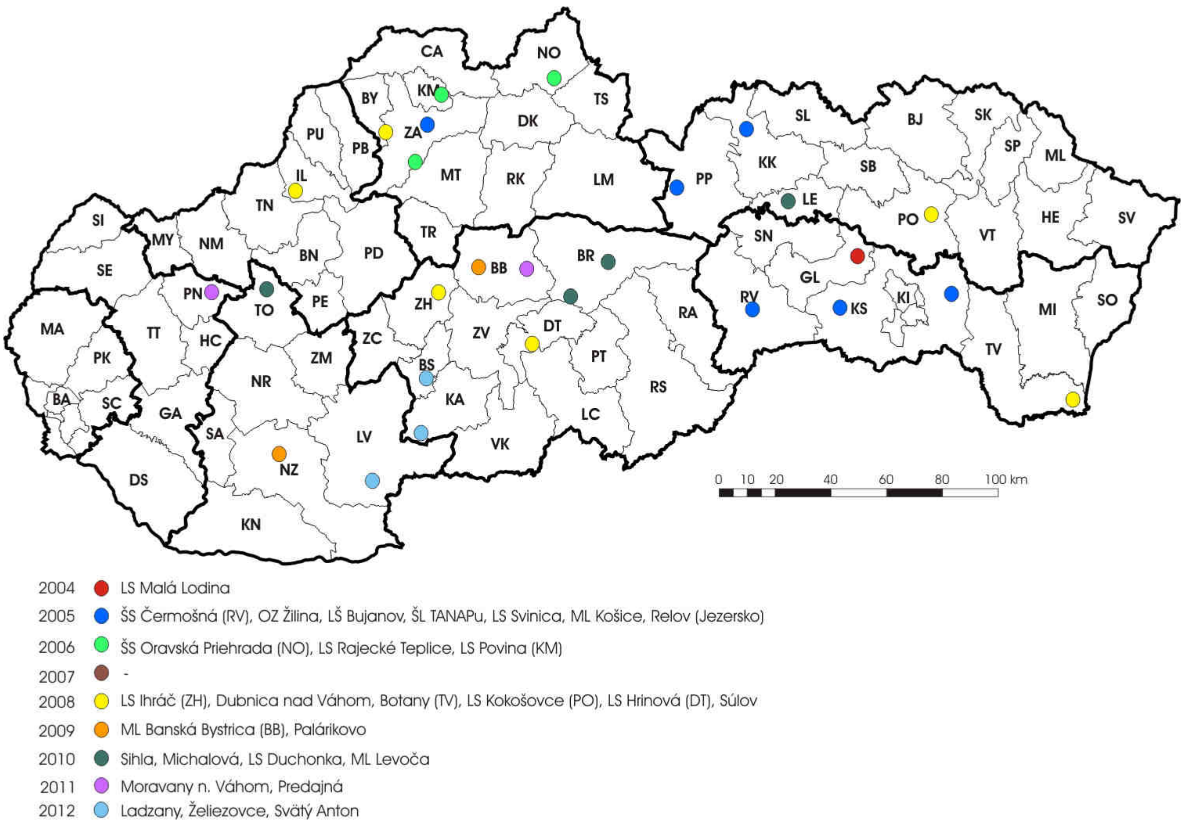

Figure 1. Occurrence of ash dieback in Slovakia from 2004 to 2012

Ash dieback was recorded for the first time in 2004 from the eastern part of Slovakia (KUNCA, 2006). Later, symptoms were observed in other localities (KUNCA, 2007, 2008; LEONTOVYČ & KUNCA, 2009), and now ash dieback has spread throughout Slovakia without any natural limits. By laboratory expertise we found several fungal pests on diseased ash trees such as *Chalara fraxinea*, Kowalski, *Phomopsis* spp. *Cytospora* spp., *Valsa* spp. The most susceptible age of ash trees ranges up to 30 years. *Fraxinus ornus* is a less common ash and so far dieback has not been found on this species, whereas it is common on *F. excelsior* (KUNCA & LEONTOVYČ, 2011). Now we can say that the disease is widespread throughout Slovakia. However there are still some stands of various ages which show no symptoms. Even at the diseased stands some trees are less damaged some stay healthy.
Trees infected by *Chalara fraxinea* are under stress and thus are susceptible to other secondary biotic pest agents. The main stem is usually attacked by bark beetles: *Hylesinus fraxini* Panzer on young stems or thin twigs, and *Hylesinus crenatus* Fabricius on older stems or thick twigs (KUNCA & LEONTOVYČ, 2010; KUNCA ET AL., 2011). Roots may be infected by *Armillaria* spp., infection may be primary, but also secondary. In some regions, browsing animals damage young ash trees, particularly deer browsing on bark. There is some concern that secondary biotic pest agents may increase in abundance on weakened trees and become primary pest agents. *Hylesinus fraxini* is considered the most dangerous secondary biotic pest agent threatening ash stands.
Currently we have ongoing work on monitoring of ash dieback and identification of pathogens. In forest nurseries and plantations we test the possibilities and effectiveness of selected fungicides. We are dealing with pure cultures, pathogenecity tests and test some factors upon the growth possibilities of pure cultures. We also want to focus on the work regarding genetic predisposition to the infection by *Chalara fraxinea*.

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Situation with Ash in Slovenia

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Three native ash species are found in Slovenia: Common ash (*Fraxinus excelsior*), narrow-leaved ash (*Fraxinus angustifolia*) and manna ash (*Fraxinus ornus*). With 3,299,144 m^3 (1.0% of total woodstock - datasource: Slovenian forestry service, 2012) common ash is the most widespread of the three in Slovenia. It is found on rich, moist, loamy soils along rivers and streams. The best common ash sites are found from lowlands up to 800 m a.s.l., while in the southern slopes in the Alps common ash can be found up to 1400 m a.s.l. Common ash often forms stands together with sycamore maple (*Acer pseudoplatanus*); occasionally, it is mixed with beech (*Fagus sylvatica*). Narrow-leaved ash represents 0.06% (211,214 m^3) of total woodstock in Slovenia. It is mainly found in sub-mediterranean region and in north-eastern Slovenia. Population genetic studies have shown that narrow-leaved ash from north-eastern Slovenia and from the sub-mediterranean region form genetically distinct clusters. It has also been observed in the field that hybrids between common and narrow-leaved ash exist in the sympatric area. This species also occurs in other parts of Slovenia, but rarely. Manna ash, with total woodstock of 1,016,577 m^3, is especially frequent and an important tree species on Kras, where it is known as a pioneer species in newly forming forests on abandoned grasslands and in Austrian pine (*Pinus nigra*) stands established in the 19th century. Additionally it is quite frequent on other extremely warm sites in southern Slovenia, while it is rare in the northern part of the country. There are 14 registered seed collecting stands of common ash and 3 of narrow-leaved ash, as well as a single clonal plantation of narrow-leaved ash.

Ash dieback was first observed in north-eastern Slovenia in autumn 2006. In the next two years the disease spread throughout the country. Common ash and narrow-leaved ash are affected, while no symptoms have yet been observed on manna ash. The severity of the disease in Slovenia seems to be higher on sites with high relative air humidity, with lower temperatures and without direct sun exposure. Ash dieback occurs on trees of all ages. Mortality is especially frequent on saplings and young trees. Apart from *C. fraxinea* fungi from genera *Armillaria* as secondary pathogens play an important role in ash decline. Lately it has been observed that ash bark beetles can cause some additional damage.

Most of the research work on ash dieback in Slovenia has been carried out at the Department for forest protection at the Slovenian forestry Institute, within the applicative project L4-2301 (Ash dieback in Slovenia and investigation of the fungus *Chalara fraxinea*), which ended in April 2012. One of the main goals of the project was to examine the effect of temperature on fungus *C. fraxinea*. Temperature assay of *C. fraxinea* colony growth *in vitro* showed that the pathogen growth was optimal between 20–22°C. No fungal growth was observed in five weeks time at 28°C or higher. In additional studies, heat tolerance of the pathogen in pure culture and in the host tissues, as well as heat tolerance of common ash saplings have been tested. Results showed that *C. fraxinea* in host tissue is yet more sensitive to heat than in pure culture. High survival rate of *F. excelsior* saplings and very low isolation success of *C. fraxinea* from diseased shoots after different water heat treatments revealed possibilities for the development of an eradication method. Furthermore, field monitoring of temperature in ash saplings in summer periods revealed that hot weather periods could obstruct the growth of the fungus in ash tissues or even eliminate it.

Laboratory experiments on the effect of selected fungicides to ash dieback pathogen have been carried out. Eight fungicides with different active ingredients (carbendazim, chlorothalonil, captan, pyrimethanil, iprodion, procloraz, fenhexamid and thiram) have been tested. Active ingredients carbendazim and procloraz were the most efficient in fungal growth suppression in pure culture as well as in suppression of apothecia formation on treated leaf petioles.

Studies of individual resistance to ash dieback pathogen were performed in the narrow-leaved ash clonal seed plantation Hraščica (46°38'45'' N, 16°16'04'' E). Crown damage was assessed each year between 2009 and 2012 at the end of July/beginning of August. In August, September and October 2011 leaf shedding of clones was assessed. In 2009, the healthiest looking clone, the most damaged clone and an average damaged clone were selected for inoculation trial. Root collars of the same three clones were inspected in 2012 for possible infections with *Armillaria* spp.The results indicate that some *F. angustifolia* clones are indeed less susceptible to ash dieback than others (significant differences between clones were observed in every crown damage assessment as well as in the inoculation trials). However, inoculation trials showed that defence mechanisms which obstruct fungal growth in wood and bark tissue may not be the most important factor for health status of the tree/crown. Positive correlation between crown damage and *Armillaria* infection confirmed honey fungi as an important secondary pest in the ash decline. Furthermore, positive correlation between crown damage and the early leaf shedding indicate that leaves, as an important infection point, could play the decisive role in the ash dieback disease development.

In 2011 monitoring plots in nine ash seed collecting stands were established and the first damage assessment completed. Average dead crown share at different locations varied between 7% and 39%. Average mortality was below 2%, 8.5% of assessed trees were infected with *Armillaria* spp. and 13% of assessed trees showed no ash dieback symptoms. However, it has to be taken into account that monitored stands were relatively old.

Experiments on apothecia development of *Hymenoscyphus pseudoalbidus* were carried out. Leaf petioles (rachises) seem to play the key role in the disease cycle. We discovered that for apothecia to mature, initial necrosis with *C. fraxinea*, enough sunlight and moisture are needed. Additionally, apothecia developed to mature phase under high relative air humidity only when there was no direct contact with water. Maximum of 102 primordia (stipes) of the fungus per rachis with average of 22 was counted. However, on average only 20 mature apothecia per rachis developed. Position of apothecia on rachis correlated with position of initial necrosis. Average sporulation was 1476 ascospores hour^{-1} apothecium^{-1}. Apothecia matured after two months at 22°C in a moist chamber. Peak sporulation took place for about two weeks. Storing rachises at low temperatures showed *C. fraxinea* growth at 1°C which makes it similar to the *Gremmeniella abietina*, i.e. psychrotolerant fungus. During the experiments several natural enemies of *C. fraxinea* were identified (e.g. fungus gnats, *Paecilomyces*).

Unfortunately, the work on the research project on ash dieback will not continue in Slovenia and financial prospects for any research in this field are limited. Damage assessments on monitoring plots at seed collecting stands will most likely continue within the cooperation with public forestry service. Furthermore, collection of seeds from trees, which show resistance to ash dieback and an attempt to produce ash saplings, which could show increased resistance to the disease, is planned together with Slovenian Forest Service and interested forest nursery owners. We are interested in collaboration on projects with research organizations from other countries. Research needs points towards development of silvicultural measures for controlling ash dieback, revealing the mechanisms of resistance to the disease and formation of clonal seed orchards for obtaining resistant trees. Findings from research projects should be put to practice and application.

Situation with Ash in Spain: stand characteristics and health condition

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Narrow-leaved Ash (*Fraxinus angustifolia*) is distributed all around Spain. This Ash was traditionally cultivated in the “dehesas” which are sparse wood pasture without scrub undergrowth and made up principally of holm and evergreen cork oak grazed by livestock. It is unique to Iberia and Morocco. Some Ash dehesas of *F. angustifolia* are still active in the center of Spain whereas the distribution of common ash (*Fraxinus excelsior*) is limited to the northern regions of the country on a wide range of soil types particularly associated with basic soils on calcareous substrates. Manna Ash (*F. ornus)* is present in small woods mixed mainly with Mediterranean oaks and pines. *Fraxinus ornus* is frequently grown as an ornamental tree for its decorative flowers of its native range.

In the spring 2010, samples were collected from both symptomatic and apparently healthy ash (*F. excelsior* and *F. angustifolia*) trees (12 trees each) in six locations (forests, dehesas and amenity situations) from the center and northern Spain. Dieback symptoms were observed on tree crowns with little wilting and premature leaf shedding, necrosis of bark and wood discoloration. The samples were placed in moist chamber and culture media with the aim to see fruiting bodies of *Hymenoscyphus pseudoalbidus.* Pathogen was not isolated from necrotic bark and wood tissues collected on symptomatic trees. The occurrence of disease symptoms failed to coincide with presence of fruiting bodies of *H. pseudoalbidus* in Spain.

During the last few weeks, information concerning dieback of Ashes in the northern and central part of Spain reached to our lab. However, confirmation of Chalara dieback in Spain does not exist to date.

Research on Chalara dieback in Sweden.

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In Sweden, research on the Chalara disease has a history of about 10 years. We have worked on defining the disease causing fungi and disease dynamics including infection biology, the metabolites and the genetic structure of the two *Hymenoscyphus* species *pseudoalbidus* and *albidus*, and the resistance level in the host *Fraxinus excelsior*. We showed that *H. pseudoalbidus* is the main disease causing agent, that *H. pseudoalbidus* and *H. albidus* are closely related but very distinct species, that *H. pseudoalbidus* has gone through a recent genetic bottleneck and that the species is not strongly structured genetically in Europe. *H. pseudoalbidus* produce the host-toxic metabolite viridiol, and also a range of other metabolites of which most are not toxic to the host. A recent publications show that there is a strong genetic component in the host population for resistance against the disease. We have also detected *H. pseudoalbidus* in seeds of *F. excelsior*, indicating seeds as a potential pathway for disease dissemination. Current projects include, among others, a genome project on *H. albidus* and *H. pseudoalbidus*, breeding for more resistant ash trees, defining the role of metabolites in the infection process, characterizing the transcriptome of the host-pathogen interaction, and further searches for the historical events that have lead to the disease outbreak.

Situation with Ash in Switzerland

A. Gross, M. Dobbertin, D. Rigling

The first record of the ash dieback disease in Switzerland originated from the year 2008. Within only four years the disease has been recorded in large parts of Switzerland. Currently, only the southern side of the Alps and a few eastern mountain valleys remain disease free. It is expected that the disease will also affect the last disease free areas within the next few years.
The Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) and the Swiss Federal Institute of Technology Zurich (ETH) are investigating various aspects of the disease and its causal agent. WSL is constantly monitoring the disease together with foresters and produce distribution maps. In addition, a Lithuanian-Swiss joint research project has been raised about the incidence of mycoviruses in epidemic and post-epidemic populations of the ash dieback pathogen *H. pseudoalbidus* and evaluation of their potential for biological control of the disease.
The group Forest Pathology and Dendrology at ETH focuses on the biology of the pathogen *H. pseudoalbidus.* Recently the reproduction biology of *H. pseudoalbidus* has been described in detail (Gross at al. in press). The mating type locus of the fungus was partly characterized and compared with *H. albidus*. Interestingly, the two cryptic and closely related species revealed different mating systems, homothallism in *H. albidus* and heterothallism in *H. pseudoalbidus*. In addition, a crossing technique for *H. pseudoalbidus* was established enabling further genetic studies of the fungus. Currently, progeny isolates of other crossing experiments including inter- and intra-species crosses with *H. albidus* and *H. pseudoalbidus* are being analyzed. Furthermore, we attempt to complete the sequencing of the mating type locus, and confirm the expression of the genes found at that locus. Moreover, a study about longevity and drought tolerance of *H. pseudoalbidus* in petioles has been conducted. Pseudosclerotia produced by *Hymenoscyphus pseudoalbidus* are extremely drought tolerant. They can survive a period of at least three months without rain and fructification can be postponed for one year, provided the conditions are unsuitable.
Using next generation sequencing techniques, the fungal communities of infected and healthy ash trees are being compared.
In a collaboration with Japanese researchers (Hosoya et al.) a population genetic comparison between Japanese and European isolates is in progress. First results show that the diversity in Japanese populations is much higher than in Europe, confirming the hypothesis that European populations went through a severe bottleneck upon introduction to Europe. However, the failure to find several European alleles in the Japanese populations questions the Japanese origin of the disease. Besides, different *Fraxinus* species, including the host tree species in Japan

Fraxinus mandshurica, are currently subject to virulence tests with various *H. pseudoalbidus* isolates from Europe and Japan in a climate chamber.
In the future, the major research needs will be to investigate the biology of *H. pseudoalbidus* in its native habitat and its interaction with the original host species *F. mandshurica*.

Ash species in Turkey

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The genus *Fraxinus*, which is a member of the Oleaceae family, has 65 species thorough the world. Even though it is found in subtropical and tropical zones the tree has the widest distirubution in the temperate zone of the Nothern Hemisphere. It is used in wood industry, furniture, music instrument manufacturing and landscape architecture due to its high growth rate, valuable wood and aesthetic value.

Turkish Forests cover 27,7% of the total lands with 21,6 million ha area. About half of the forest land area consists of hardwood species. Ash, which is one of the most important dedicious tree species in Turkey, covers approximately 14,410 ha high forest area, spreading about 0.06% of all Turkish forests.
There are three species and six subspecies of *Fraxinus*; *Fraxinus angustifolia (F. angustifolia* subsp. *oxycarpa), F. excelsior* and *F. ornus (F. ornus* subsp. *cilicica)* distrubuted in Marmara, Black sea, Aegian and Mediterranean Region of Turkey. *F. angustifolia* called as "narrow-leaved ash" has world's largest distrubution in Turkey and it covers 12.500 ha of total ash forest area in the country.
F. angustifolia is a indigenous tree species that has been introduced in the plantations in Turkey. *F. angustifolia* is found sometimes in pure stands along the alluvial delta on Sakarya stream of the East Marmara Sea Region but more frequently found mixed with other broadleaves including *Acer, Alnus, Carpinus, Juglans, Quercus, Ulmus, Platanus, Populus, Salix, Sorbus* and *Tilia species,* as well as various small trees and shrubs including *Cornus, Corylus, Crataegus, Ligustrum, Malus, Pyrus, Ramnus, Rosa, Sambucus, Tamarix* and *Viburnum* species.

F. angustifolia grows quite well on moist, rich clays in bottomlands and moist, well- drained soils in highlands of Turkey. If the groundwater can reach on soil level during February and April, the species can resist temporary flooding. It prefers loams, sandy loams and sandy clay loams with pH in the range 6 – 8 and sub- soils are usually alluvial. *F. angustifolia* requires temperate climates with the mean annual rainfall from 400 mm to 800 mm. It is known as a light-demanding species and has short rotation period, which is about 40 years in Turkey. Average annual volume increments per ha (m3/ha/y) are 23 in plantations and 15 in natural stands.

Although its natural stands are confined to bottomlands known as marginal areas for tree growth, Adapazarı- Süleymaniye is the biggest bottomland for *F. angustifolia* where it grows best in Turkey. It has a total area of 5,150 ha according to forest management plan. Like many tree species in Turkey narrow- leaved ash is also not in good conditions due to fires, overgrazing and excessive cutting caused for centuries. For this reason 95 percent of the ash stands have been converted to plantations for the past fourth years in Turkey.

F. excelsior is second common ash species in Turkey. It can reach heights of over 40 m with a girth of up to 6 m. *F. excelsior* grows well on calcareous soils and requires the soil to be rich in humus and nutrients. It is known as a pioneer tree, which has high tolerance against most environmental factors except a shortage of light. The species has been defined as an ornamental plant due to its beautiful flowers, foliage, and interesting crown shape.

Third ash species grown in Turkey is *F. ornus.* It occurs naturally on warm south- facing slopes in broadleaved and mixed forest, typically forming groups and small stands in association with *Quercus* spp. and chestnut as well as hornbeams and maples. It is rarely found in mixtures with conifers, except some species of junipers as an understorey species with firs and beech in Turkey. *Fraxinus ornus* subsp. *cilicica,* the Taurus Flowering Ash, is an endemic tree that is located in the Taurus Mountains of Southern Turkey. *F. ornus* usually grows from 8 to 20 m height. The tree prefers sunny southern slopes in karstic areas and grows between 350 and 1,500 m in Turkey.

Researches carried on Ash disease in Turkey are quite limited. We performed a primary study in Turkey on *F. angustifolia* subsp. *oxycarpa* located in Hendek Süleymaniye, Kaynarca Acarlar and İğneada Longoz, as well as forest nurseries in İğneada, Hendek, Eskişehir and Düzce, where *F. angustifolia* subsp. *oxycarpa* or *F. excelsior* seedling are being produced. Trees and seedlings were surveyed for the presence of dieback and canker symtoms. Fungal isolations were done in laboratory conditions and fungal species were identified based on morphological characteristics and sequencing of ITS regions of DNA.

As a result, totally 1268 isolates were obtained from 410 symptomatic samples. The most common fungal genera were; *Phoma* Sacc. and *Phomopsis* Sacc. & Roum., *Bionectria* Speg, *Daldinia* Ces. & De Not., *Pestalotiopsis* Steyaert and *Alternaria* Nees. *Chlara fraxinea* was not reported in the area we studied.
In near future we are planning to run a master thesis on existence of fungal species, with the special emphasis of *Chlara fraxinea* and water molds, including *Phytophthora*, *Pythium* and *Phytopythium* on ash, alder and beech species.

Key words: Ash, Fraxinus, dieback, Turkey

Situation with Ash in Eastern Ukraine: stand characteristics, health condition, ongoing work and research needs

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During the last years, severe dieback of common ash (*Fraxinus excelsior*) caused by *Hymenosyphus pseudoalbidus* is observed in most European countries. Until 2010, there was little concern regarding ash health condition in Ukraine, as no strong dieback symptoms were reported. First some morphological symptoms of ash decline were observed in Eastern part of Ukraine during 2010-2011 years: uneven flushing, occasional shoot necrosis, discoloration of wood and premature leaf-shedding.

Forest ash stands of eastern Ukraine represent mixed deciduous forests with predominance or participation of *F. excelsior* L. For monitoring of ash stands condition nine study sites were done in three regions of East Ukraine including different provenance tests (five ecology-geographical populations) of mature ash stands. These sites represented ash stands either 10 to -80-year old with different crown condition. The various degrees of ash crown decline from 20 to 80% were observed in different sites during 2010-2012. Also, we have a three monitoring sites where no ash dieback symptoms were found.
We have investigated fungal communities in necrotic and healthy-looking shoots, and in petioles of leaves that were shed during previous vegetation season. In addition, health condition of different ash provenances was visually assessed on nine sites (24 test trees in every plot). In total, 472 samples from 9 monitoring sites were studied. The detection of fungi has been accomplished using molecular methods with ITS fungal primers and *C. fraxinea*-specific primers. In 2011, for a first time in Ukraine the fungus *H. pseudoalbidus* was detected, but it was determined for 6 monitoring sites in three regions of East Ukraine with difference occurrence. Also, we collected 6 isolates of *H. pseudoalbidus* from infected branches and apothecia.
Now, our research interests include the work on identification of *H. pseudoalbidus* in other regions of the Ukraine, the pathway of spread this ash disease in our country, collection isolates from infected shoots and apothecia on ash petioles and to compare them for pathogenicity towards ash seedlings, as well as on the genetic diversity of causal agent and characterization of this pathogen using PCR-based techniques.

Situation with Ash in Western Ukraine: stand characteristics, health condition, ongoing work and research needs

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During the last five years on the territory of Western Ukraine there has been observed a dieback of ash tree stands.
There are generally 9837.5 hectares of ash tree stands in Lviv and Ternopil regions. Except this, here are created plantations of exotic species of ash (*Fraxinus americana* and *Fraxinus pennsylvanica*) –which occupy 54.3 hectares.
Ash forms pure and mixed tree stands. Its total area of plantations in Lviv region makes 3515 hectares, 61% of which is occupied by the tree stands, consisting of ash (less than 5 units), and only 7.8% are pure ash stands. In Ternopil region – the total area of ash tree forests makes 6322.5 hectares (about 70% of which - are mixed tree forests with 5 units of ash). Most frequently ash is mixed within oak forests.
Currently in Western Ukraine clear sanitary cuttings in the dying ash tree forests are being conducted. The largest areas of clear sanitary cuttings were appointed in mixed tree stands including ash (from 4-to 7) and 8-10 units. However, tree stands which contain 4 or less units of ash dry and dieback.
According to the data of Forest Service, the reasons of ash dieback in Lviv region are flooding, root rot and xylophagus insects. In Ternopil region the main causes of ash drying are bacterial canker and root rot.
After examining ash tree forests aged from 55 to 77 in Western Ukraine the most typical types of ash damage such as dead branches, dead top of branches, necrosis on branches and on stem, cankers on trunk (*Pseudomonas savastanoi* pv. *fraxini*, *Nectria galligena*) and root rot (*Armillaria spp., Ganoderma lipsiense*) were found. Stem rot is caused by the following species: *Laetiporus*

sulphureus, Fomes fomentarius Oxyporus populinus, Trametes ochracea, Trametes versicolor.
There was a very small percent of healthy ash trees.
Because of the increase of dieback of ash areas in Western Ukraine, it is necessary to continue the study of the causes of ash mortality in our region.

Situation with ash in the United Kingdom: stand characteristics, health condition, ongoing work and research needs

Steven Hendry

European ash (*Fraxinus excelsior*) is the third most common broadleaved tree species within British woodlands, occupying an area of around 130, 000 Ha (approximately 5.5% of the total forest area). However, it displays marked regional differences in abundance being much more numerous in the southern than the northern half of the country. Outwith woodlands, it is a prominent feature of the landscape with an estimated 11.8 million ash growing as individual trees, along boundaries & rivers, and in small groups throughout the country.

Ash dieback due to infection by *Chalara fraxinea* was first detected in the UK in February 2012 in a consignment of ash trees sent from a nursery in the Netherlands to a nursery in the English county of Buckinghamshire. Since then, diseased plants have been found at a number of recently-planted sites in both England and Scotland: all of the sites concerned had received stocks of young ash plants from nurseries within the past five years. In parallel with these findings, further cases of *C. fraxinea* infection have also been confirmed in plants at a number of different nurseries. In October 2012, a small number of cases of ash dieback which did not appear to have any association with recently supplied nursery stock were confirmed in mature ash woodlands in the southeast of England (East Anglia).

Since the beginning of November 2012, government agency staff have been carrying out a rapid UK-wide survey for signs of the disease mainly within established woodlands. Member of the Concerted Action will be updated on the latest findings from this survey at the Vilnius meeting.

Currently perceived research needs in the UK include: refinement and development of methodologies for rapid detection of *C. fraxinea*, further investigation of the etiology, pathology and epidemiology of the pathogen (e.g. population analysis), and the development of disease adaptation strategies suited to the UK's circumstances.