Danish Workshop meeting on Chalara, Skovskolen, Denmark 2017,

Summary report from a meeting of European experts**:** 23-25 August 2017,

'Fighting ash dieback with new and old tools' ,

Report: Gerry Douglas Teagasc

This workshop was held in the framework of the project HealGenCar, which involves a collaboration among mainly Scandinavian researchers who are involved in breeding ash trees for resistance to ash dieback disease caused by the fungus *Hymenoscyphus fraxineus.* The workshop had invited 29 participants from nine countries.

Note: all common ash trees (*Fraxinus excelsior*) appear to be susceptible to ash dieback disease. In environments with a high disease pressure, a small percentage of trees appear healthy and their growth and development is generally unaffected. These trees are referred to as 'tolerant' or 'resistant' in this report. Trees which lose most of their leaf canopy, do not thrive and may eventually die, are referred to as 'susceptible'.

Summary information from presentations:

Lars-Göran Stener[1,] Michelle Cleary[2]

Ash "breeding" projects in Sweden

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In Sweden they have a long term breeding strategy for important species such as pines and spruces and funding for these is secured. The funds are less secured for breeding of more minor species on a short term basis, such as ash and wild cherry.

Ash dieback was first observed in 2001 in Sweden and ten years later 20% to 30% of trees in forests were severely damaged and mortality is 8% per year throughout Sweden. Studies indicate that ash may become extinct in Sweden and regarding its conservation status it is now in the 'endangered' category. Resistance to ash dieback is the basis for tree selection and further breeding. Before ash dieback they had 106 selected plus trees from 27 different stands. Analysis of their selected plus trees in clonal seed orchards, showed that the 10 least damaged clones in 2006 remained healthy in the following three years. They showed that the higher growth trees were correlated with less tree damage and the more tree damage due to ash dieback, the more canker was correlated. In summary, they found that resistance in clonally propagated material remained resistant over many years, there is a high level of genetic control in resistance, there was no significant site effects regarding levels of resistance.

For long term breeding for resistance, they estimate that they need 300 to 500 trees as a base population. They have selected 508 healthy trees in 120 stands and they are being monitored for tree health yearly. Co-operation is needed regarding; the exchange of resistant clones, establishment of joint field tests to determine genotype, environment (GXE) and climate effects as well as the need for a standard scoring system for disease levels. In their field progeny trial (2014) they collected seeds from 6 tolerant trees and 7 susceptible trees and progeny showed a 13% mortality after 3 years.

Jonathan J. Stocks[1,2], Richard J. A. Buggs[1,2], Steve J. Lee[3]

A first assessment of *Fraxinus excelsior* (common ash) susceptibility to *Hymenoscyphus fraxineus* (ash dieback) throughout the British Isles

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In the UK, a trial was established in 2013 on 14 sites in infected areas consisting of 150,000 saplings in 650 plots to identify potentially dieback tolerant trees by their absence of symptoms. There is an Irish component in this trial consisting of approximately 13,000 trees from two provenances in Clare and Donegal. First results on two intensively monitored sites give an average mortality of 18% due to ash dieback and an average 3% of the trees which are still healthy. Scottish and Irish sources of saplings appeared relatively less damaged compared to other sources from England. Further monitoring is required on an annual basis to exclude the possibility of selecting trees which had escaped disease by chance. Their research is now concentrated on finding genome wide SNP markers to distinguish resistant and susceptible trees at the DNA level.

Rasmus Enderle[1] and Barbara Fussi[2]

A (too?) early approach in breeding for resistance against ash dieback in southern Germany

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Ash dieback was first observed in Bavaria in 2005/06. They have collected shoots from resistant trees, propagated them by grafting and found that resistant individuals remained resistant in field trials.

They have tested progeny from resistant trees which were planted out in 2015 and the second best selected mother tree (regarding level of resistance) gave the most resistant progeny / family of seedlings with resistance.The levels of resistance among various family progenies was similar on two different sites, indicating that the resistance is under high genetic control. Their experience indicated that selecting resistant trees just five years after the arrival of ash dieback was too early to select for strongly resistant trees. A discussion followed which concluded that resistant trees should be best selected within ash stands and that the resistant individuals would be those trees with full crowns of leaves, which are surrounded by nearby trees which are heavily diseased, dead and / or dying.

Thomas Kirisits[1,*], Christian Freinschlag[1,2], Raphael Klumpp[3], Heino Konrad[4] and Gregor M. Unger[4]

Resistance of ash to *Hymenoscyphus fraxineus*: evidence from Austria

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Ash dieback was first confirmed in Austria in 2005 and ten years later there was extensive damage to forests. They have monitored disease progression in three seed orchards using a scoring system which assesses damage in the top, middle and bottom thirds of a tree crown. In 2017 there was a 22% increase in crown damage over five years earlier. There was no tree mortality in 2012 but trees were dying in 2015 and the dead trees were those with the highest levels of crown damage in earlier assessments. They have progeny tests underway using seeds from resistant trees and in the first year (2014) there was little damage to foliage, however mortality of up to 2% was observed in 2017. All eight progenies in their trials performed similarly on two different sites and the damage level in the progeny corresponded to the damage level of the mother trees, indicating a strong genetic component for resistance.

Gregor M. Unger[1,*], Heino Konrad[1], Thomas Kirisits[2], Katharina Schwanda[3], Thomas L. Cech[3], Gernot Hoch[3], Silvio Schüler[4] and Thomas Geburek[1]

"Ash in distress": the conservation and resistance breeding programme for *Fraxinus excelsior* in Austria

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This group has inspected 556 ash sites in Austria and selected 338 sites where selection of resistant trees could be done. Healthy trees were selected with have a dbh of 30 cms; no crown damage, with dense foliage and an absence of collar rot. For selective breeding they have harvested seeds from 580 selected mother trees and 610 progeny saplings have been planted out in the nursery. In addition they have placed inoculum of the ash dieback fungus among the trees so as to increase the disease pressure in order that resistant individual can be more securely identified as opposed to trees which have escaped infection by chance. They plan to identify the father trees of the most resistant progenies by doing paternity tests.

Their objective is to set up seed producing orchards using the most highly resistant mother and father trees.

Caterina Villari(1), Arnaud Dowkiw(2), Rasmus Enderle(3), Marjan Ghasemkhani(4), Thomas Kirisits(5), Erik Kjaer(6), Diana Marčiulynienė(7), Lea McKinney(6), Berthold Metzler(3), Facundo Muñoz(2), Lene Rostgaard Nielsen(6), Alfas Pliūra(7), Lars-Göran Stener(8), Vytautas Suchockas(7), Luis Rodriguez-Saona(9), Pierluigi Bonello(10), **Michelle Cleary (4)** (1) Warnell School of Forestry & Natural Resources, University of Georgia (Athens, Georgia) 180 E Green Street, 30602 Athens, Georgia, United States. (2) Institut National de la Recherche Agronomique (INRA) (Ardon, Orleans) 2163 Avenue de la Pomme de Pin, 45075 Ardon, Olreans, France. (3) Forest Research Institute BadenWurttemberg, Department Forest Protection (Freiburg) Wonnhaldestrasse 4, 79100 Freiburg, Germany. (4) Swedish University of Agricultural Sciences (SLU), Southern Swedish Forest Research Centre (Alnarp) Sundsvägen 3, 23053 Alnarp, Sweden. (5) Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences, Vienna (BOKU), Peter-JordanStraße 82, 1190 Vienna, Austria.(6)Department of Geo sciences and Natural Resource Management, University of Copenhagen (Copenhagen) Rolighedsvej 23, 1958 Frb Copenhagen, Denmark.(7)Lithuanian Research Centre for Agriculture and Forestry, Institute of Forestry (Girionys, Kaunas district) Liepu 1, LT53 101 Girionys, Kaunas district, Lithuania.(8)SKOGFORSK - The Forest Research Institute, Ekebo 2250, 26890 Svalöv, Sweden.(9)Department of Food Science and Technology, The Ohio State University, Parker Food Science and Technology (Columbus, Ohio) 2015 Fyffe Road, 43210 Columbus, Ohio, United States.(10)Department of Plant Pathology, The Ohio State University, 201 Kottman Hall (Columbus, Ohio) 2021 Coffey Road, 43210 Columbus, Ohio, United States. (1) Email: Michelle.Cleary@slu.se

Advanced phenotyping using FT-IR distinguishes disease resistance in *Fraxinus excelsior* against *Hymenoscyphus fraxineus*

Researchers in Sweden and other countries have collaborated in developing a new system of phenotyping ash trees for resistance based on a FT-IR (Fourier-transform infrared) technology. It involved making chemical fingerprints of well characterized resistant and susceptible sets of trees and developing a computer model to distinguish each set. The analysis was done on purified phenolic extracts of leaves and phloem tissues. The model showed that phloem (stem) extracts were better than leaf extracts and also that the model could discriminate between randomly selected resistant and susceptible trees in blind tests. This technique offers the prospect of identifying resistant trees based on their chemical fingerprints.

Gerry Douglas and Miguel Nemezio Gorriz

Research on ash dieback in Ireland Teagasc

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The importance of ash and the current situation regarding ash dieback in Ireland was outlined. Disease pressure in Ireland is currently too low to allow any selection of dieback resistant trees. Therefore trees of Irish provenance that exist in trials abroad such as in the UK, Belgium, Germany and France would be sources for identifying trees with potential tolerance to dieback disease because the disease pressure in those countries would be at a level to allow selection. Efforts to acquire disease resistant plant material, to build up a cohort of resistant individuals, for generating seed producing orchards was described. Details were provided on the research project for propagating material and the screening of 1000 genotypes for screening in trial sites in Lithuania in conditions of high disease pressure.

Asiatic species of ash such as *F. mandshurica* are known to be resistant to ash dieback disease and transferring genes from that species into common ash would confer disease resistance. In addition *F. mandshurica* is resistant to other pathotypes of *H. fraxineus* which are not currently in Europe, and also to the Emerald Ash Borer. The experiments on crossing common ash with resistant Asiatic species and the production of putative hybrid plants was described as an additional tool for transferring genes from these species into common ash.

Lea Vig McKinney, Chatchai Kosawang, Ghanasyam Rallapalli et al.

Transcriptome profiling of tolerant and susceptible ash

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A small fraction (1-3%) of ash tree populations exhibit a low susceptibility to ash dieback disease, however the mechanism of this tolerance is unknown. To determine the mechanism, the transcriptome of tolerant and susceptible ash (*F. excelsior*) as well as that of related species (*F. mandshurica*) were exposed to the fungus in chambers and the expressed genes were then analysed. They found that there was no single gene expressed which could discriminate the resistant from susceptible trees during the first phase of the infection process. However, several genes involved in pathogen-host interactions and several transcription factors were differentially regulated during leaf infections in trees of varying susceptibility.

Urszula Wasileńczyk, Anna Pasławska

Assessment of the effectiveness of cryotherapy and thermotherapy in the fight against pathogens

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This institute is making collections of ash trees' seeds throughout the geographic range of Poland in a conservation project. Before storage, the seeds were tested for the presence of microorganisms including the ash dieback fungus (*Hymenoscyphus fraxineus*). They found that treating seeds at high temperature could inactivate the ash dieback fungus. They also showed that ash seeds with a moisture content of 10% could be stored in liquid nitrogen (-150 C° for very long periods.

Chatchai Kosawang[1], Erik Dahl Kjær[1], Lea Vig McKinney[1], David Collinge[2] and Lene Rostgaard Nielsen[1]

Fighting Ash Dieback with Endophytes

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Ash shoots contain many microorganisms internally as harmless endophytes. The range of species of microorganisms in resistant and susceptible ash trees was examined from twigs of each group of trees. The pathogen causing ash dieback *Hymenoscyphus fraxineus* was found to be present in both resistant and susceptible plant material. More fungal species (endophytes) were detected in resistant clones when compared to susceptible ones. They also were able to identify fungal isolates which were either specific to or significantly abundant in resistant clones. They are now using various pairs of fungal isolates to determine how they can interact and inhibit each other with the view to identifying fungal isolates that may have potential to control the ash dieback fungus biologically.

Maryam Rafiqi[1] and Mark McMullan[2]

Effector complement of the ash dieback pathogen *Hymenoscyphus fraxineus*

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Over 10% of the world's species are vulnerable to extinction due to fungi. Plant cell processes facilitate the growth of fungal pathogens and fungal pathogens produce effector molecules which facilitate the development of the fungus to invade the host tree so it can complete its life cycle. The suite of effector genes in the pathogen *Hymenoscyphus fraxineus* was examined and it was estimated that 10% of genes of *Hymenoscyphus fraxineus* consist of effector genes. They plan to determine which of these constitute functional genes associated with the processes of host infection.

Lene Rostgaard Nielsen[1], Chatchai Kosawang[1], Lea V. McKinney[1], Søren Barsberg[1], Erik D Kjær[1], David Collinge[2] *et al.*

Presentation of a new project: Unravelling ash-dieback resistance: insights from single-cell analysis of tolerant and susceptible ashes across evolutionary divergent clades

1. IGN, University of Copenhagen 2. PLEN, University of Copenhagen

It is known that different sections in the genus Fraxinus respond differently to infection by ash dieback fungus (*Hymenoscyphus fraxineus*) and that species from Asia show a higher level of resistance. Although *F. mandshurica* can produce ascospores and little leaf damage it does not enter the stems nor kill trees. The planned research is to study the processes of infection in different species and identify the molecular defense mechanisms involved in resistance and characterizing any differences between resistant and susceptible trees.

Ash breeding projects in Denmark

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An overview on selection and breeding for resistance in Denmark was presented. Among two infected clonal sites consisting of 39 clones, 63% of all genotypes have died since 2007 and only 1 – 2% remain healthy. It was determined that early leaf senescence in autumn was associated with higher levels of resistance. In addition the heritability of mother to offspring correlation was very strong. Similarly the correlation of mother tree to vegetatively propagated offspring was also very high indicating the strong genetic component to disease resistance. Seeds have been collected from 101 resistant trees and their offspring is now being tested on 14 sites throughout Denmark. In addition, they have established four clonal seed orchards consisting of 113, 121, 40 and 40 resistant genotypes in each respectively. The resistant trees selected were in mature stands and also in younger pole stage stands.

Barbara Fussi, Ralph Jenner, Roland Baier

Genetic diversity of resistant ash in a German provenance trial and seed testing for gene conservation

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Ash dieback disease has been monitored in provenance trials from 2005. Mortality was zero in 2011 and rose to 18% in 2015 and 29% in 2017. Healthy resistant trees were recorded at 7% in 2011 and this is reduced to 2.9% in 2017. All provenances have some healthy individual trees ranging from 52% to 16% depending on the site. Analysis of genetic markers showed a slight tendency of higher observed heterozygosity in cohorts of healthy trees. They are now aiming to combine all healthy trees in a breeding seed orchard.

The also studied the viability of ash seed stored from 1994. Germination was 50% after 10 – 15 years of storage but did not decrease further thereafter. Stored seeds may have a use as a reservoir of genetic diversity in case the dieback fungus increases in its pathogenicity.

Audrius Menkis, Kateryna Davydenko, Astra Zaluma* Jan Stenlid, Rimvys Vasaitis

Testing ash for resistance to dieback: an amateurish approach

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Gotland is a large island in the Baltic Sea which has become infected with ash dieback disease on ash trees and Dutch Elm disease on elms. Their project aims to select 100 dieback resistant ash trees which are native to Gotland within severely damaged stands focusing on Natura 2000 sites. Seeds were collected from 123 such trees and progeny saplings planted out for further screening. The planted progeny saplings failed but 1032 naturally regenerating saplings were selected for disease tolerance within diseased stands and planted out for further screening in 2017. So far less than 10% of these trees show disease symptoms but they will be monitored for susceptibility and further selection in the future.

Jan Stenlid[1], Rajiv Chaudhary[1], Michelle Cleary[2], Malin Elfstrand[1].

In search for ash trees tolerant to ash dieback: Mapping approaches using multiplex PCR of gene space markers.

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First infection of ash dieback was confirmed in 2003 and by 2015 the disease has had a devastating impact on all forest areas of Sweden. Over 500 trees have been selected which show a high level of tolerance and will be used to produce seeds in seed orchards after grafting. From these they have selected 200 resistant and 150 susceptible trees to find genetic markers for each condition using a multiplex PCR approach of 30 – 50 genes. The resistance/susceptible phenotypes will then be associated with the genetic markers produced. The ultimate goal is to develop genetic markers that can predict field tolerance to ash dieback disease.

Yuri Baranchikov

Emerald ash borer in European part of Russia: 2017 situation update

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Russia has four native species of ash in their territories *F. excelsior, F. mandchuria, F. angustifolia* and *F. chinensis*. The tree borer beetle *Argilus planipennis* has been found killing trees in the Moscow area since 2003. In N. America this beetle has eliminated millions of ash trees in 29 states with a cost of $1.5 billion p.a. and in two provinces of Canada. It has potential to spread to Western Europe. Dendrochronology examinations showed it invaded the Moscow area first in 1997. It has moved into many regions especially along highways and has been recorded in Vornezh province, which shares part of its borders with eastern Ukraine. However, in the past three years the population in the Moscow area has fallen and this may be due to the emergence of a local polytrophic parasite *Spatthius polonicus* which may be useful in regulating the buildup of damaging levels of the Emerald Ash borers This beetle borer is spread inside infected ash logs used for firewood and the threat of its introduction to Ireland would be mainly by that route.

Visits to experimental field trials in Denmark

Site 1. Valby Hegn

The forest site at Valby Hegn was used to trace the fate of seedlings that had been naturally dispersed from healthy and unhealthy trees regarding ash dieback disease. The stand consisted of open pollinated progeny from a clonal seed orchard (with 8 genotypes) and was planted in 1958. They sampled 469 reproductively mature trees as potential males and females for the 399 sapling that were sampled in a forest transect as potential progeny from those identified parent trees.

In addition they collected 50 seeds from each of 11 mother trees as part of the analysis. Using molecular markers and paternity analyses they could identify progeny that were derived from healthy and diseased parent trees. 44% of saplings could be attributed to sets of parent trees and 50% of seeds could be attributed to sets of parent trees. Parentage analysis showed that 91% of pollen is dispersed within the forest stand at distances of up to 120m, while seeds can be dispersed up to 80m from parent trees. The overall result was that the more healthy mother trees produced more seeds and gave rise to healthier progeny in this natural regenerating forest. These important results have shown that the reproductive fitness of mother trees that are relatively unaffected by ash dieback disease gave rise to relatively healthy progeny in the form of saplings that were naturally regenerating in the forest.

Site 2. Tjaerby

The site at Tjaerby consists of 131 grafted clones, selected for high tolerance to ash dieback disease with 8 ramets per clone and field established in 2017. They also planted out 1404 unselected sapling plants as controls in the trial to monitor the levels of disease tolerance with reference to the selected clones using a five point scale of zero to four. This trial is also replicated at another site in Staerkende.

Site 3. Slangerup

This trial site has been planted with progenies that were derived from controlled crosses of the most healthy individuals, within their seed orchards, with unhealthy individuals regarding ash dieback disease. Controlled crosses were made in 2012 and the resulting sapling families were planted out in 2016. They genotyped 200 progeny seedlings using microsatellites and for 142 of the seedlings, they identified the fathers and mother trees. This means that they now have pure genetic families of the various crosses. They are now planning to find genetic markers for dieback resistance in the form of SNPs. They will also use this material to test / confirm the value of previously described markers associated with susceptibility and resistance to ash dieback disease.