

REPORT on FraxNet Meeting Malmo 25-28 August 2026

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Fraxnet is a project in the EU programme Euphresco which supports coordination and collaboration in the area of phytosanitary research, and to maintain itself as a strong, long-term network of research stakeholders.

Introduction

Participants included the principal individuals and Institutes which have been working on studying ash dieback (ADB) and Emerald Ash Borer (EAB) including: Denmark, France, Switzerland, UK, Sweden, Ireland, Germany, USA, Italy, Austria, Poland, Ukraine, Latvia, and Norway. For some countries, presentations were made by more than one group. We summarise below the presentations from a representative selection of the presentations in each of the three sessions.

Many of those presenting research results had summarised *the state of the art regarding the nature of the Ash Dieback fungus, the genetic nature of resistance in ash trees, the recurrent selection of healthy trees and the various approaches that are being used for breeding.*

In a similar way we have summarised the state of the art regarding Emerald Ash Borer regarding its nature, detection, dissemination, expression of resistance in ash trees and interaction with ADB. Summaries for each pest are given below.

Summaries of science and seed orchard and deployment approaches of tolerant ash in Denmark and possible relevance to Ireland

Summary of science regarding ash dieback (ADB)

1. The disease is caused by the fungus *Hymenoscyphus fraxineus* (Ascomycota)
2. Ascospores are released from the rachises (petioles) of fallen leaves in the leaf litter of previous years
3. The fungus can remain dormant for many years as a black pseudosclerotia¹ layer on rachises and fructify when conditions are favourable
4. The fungus alternates between a sexual reproductive phase on fallen leaves with ascospores dispersed mainly in summer and a damaging asexual phase mainly in the phloem of living ash trees

¹ Pseudosclerotia are a compact mass of hardened fungal mycelium that incorporates host material (like wood or fruit tissue) or soil particles. They act as a survival stage and let fungi wait out harsh conditions like winter or dry spells in the soil.

5. The fungus may be isolated and cultured from necrotic stem lesions and used to re-inoculate trees to determine tree sensitivity / resistance by measuring the length of lesions as a quantitative character
6. Natural infection occurs when the ascospores germinate on the leaves and stem lenticels and penetrate the plant tissue, starting the asexual phase.
7. The fungus grows inward from the leaves down into the leaf rachis (petiole) and into stems causing long necrotic lesions
8. At ground level spore concentrations are highest in woodlands and spores may germinate and infect the base of stems via lenticels
9. Ash trees of all ages are affected by the fungus
10. Artificial inoculation of trees has shown that some trees can limit the spread of the fungus among trees that had been selected as field resistant. However, other trees succumbed to artificial inoculation but had shown a natural resistance to natural means of infection.
11. In ash populations, individual trees may be found in a healthy condition regarding having almost a full crown of leaves and an absence of necrotic lesions on trunks, branches or at the base of trees at ground level. The frequency of healthy trees in natural forests and plantations has been reported in the range of 1 to 3% in several studies
12. Tolerance to ash dieback has been shown to be genetically transmitted to offspring both in controlled conditions and also within natural woodlands
13. Estimates of heritability indicate that it is feasible to breed trees for tolerance to dieback by forward selection within progenies that are derived from healthy mother trees.
14. Selection is also feasible in progeny derived from clonal seed orchards which consist of mother trees selected within heavily diseased stands
15. Seeds may become available somewhat faster from clonal seed orchards (5-10 years) compared to seedling derived orchards (10-15 years)
16. Seed derived plant material (from progeny trials and clonal seed orchards) consist of known mother trees but father trees from outside the trial material contributes at least 50% of the pollen, which can travel and pollinate from distances up to 1 km.
17. Seed derived plant material (from progeny trials and clonal seed orchards) may be bulked up by vegetative propagation.
18. Several studies have reported some tree chemicals which are involved in restricting / inhibiting the growth of cultures of *Hymenoscyphus fraxineus* in vitro and also in stem tissues.

19. Molecular studies have found statistical associations between SNPs² (and other markers) and tolerance to ADB which have re-confirmed the genetic basis for tolerance.
20. The basis for tolerance while genetically based is polygenic and involves the expression and interaction of many structural and regulatory genes, perhaps thousands.
21. Knowledge of the molecular and chemical mechanisms which confer tolerance to ADB is not essential to breeding trees for resistance because the morphological expression of tolerance (healthy trees) is easily identifiable and selectable.
22. Success in breeding for ADB resistance depends on:
 - a) the genetic diversity present in the base populations in which healthy trees are selected
 - b) The selection pressure that is present on the population, where varying levels of tolerance is expressed among those trees and where the most tolerant individuals can be identified and used for breeding.

Summary of science regarding emerald ash borer (EAB)

1. EAB infects and kills all *Fraxinus* species in USA though there are differences in the susceptibility among species with *F. pennsylvanica* being most susceptible.
2. Asian species of ash appear to be resistant to EAB³
3. *F. excelsior* is susceptible to EAB but is less susceptible than US species, this tolerance is linked to a chemical reaction which interferes with larval development
4. Natural rate of expansion of a beetle population is approximately 10 km / year.
5. In the USA the pest may be introduced on logs containing larvae and by long distance transport of adult beetles on vehicles - 'hitchhiking'
6. The responses of trees affected by ADB are being studied in relation to their susceptibility to EAB and the effects on beetle development and tree vitality
7. A very small proportion of ash trees in the USA show resistance / tolerance to this pest and are called 'lingering ash' - trees that can only be detected when almost all the ash trees in a stand have been killed
8. It is very difficult to detect the initial infestations visually.

² An SNP is single nucleotide polymorphism, which is a variation at a single position in a DNA sequence among individuals. To be considered a SNP rather than a rare mutation, the variation must be found in at least 1% of a population.

³ See Plumb WJ et al <https://nph.onlinelibrary.wiley.com/doi/full/10.1002/ppp3.70069>

9. It is too late to engage in attempts at pest eradication when typical 'D' shaped holes are evident in infested trees. The holes represent the exit ports of the mature beetles which had developed as larvae and pupae under the bark for several of the preceding years.
10. Measures in the USA to restrict the spread of the insect, such as sanitation felling, as well as restrictions and advice on firewood movement, have failed.
11. Breeding in the USA of ash species for resistance to EAB is at an early stage and involves detecting 'lingering ash' trees and using them for breeding as well as efforts to hybridise American species with Asian species⁴ (e.g. *F. mandshurica*) so that resistance genes can be introgressed in the long run.

Summary of deployment approaches of tolerant ash in Denmark and possible relevance to Ireland (the reader is directed to the full report and the Denmark tour handout for more detailed outline of the Danish approaches)

- Ash selection in Denmark is designed to keep high effective population number - would it have been relevant to select stronger within and between families to get higher gains?

Comment: This is one of the most common considerations in tree breeding programmes. The Danish approach is forward selection by seed collection from putatively tolerant parents in the forest, establishment of a BSO, rouging to remove diseased trees and reduce the breeding population and provide space for crown development and seed production as the next bullet outlines.

- The plan is to continue strong forward selection down to 350 trees during the next 10 yrs to increase gain, allow selection for other criteria and ensure abundant seed collection.

Comment: Assuming plant spacing was close to 2 x 2 m for c 3500 trees, the final 350 trees will have a spacing of c 40 m²; c 6 x 6 m which seems adequate for seed production. With a BSO selection for traits such as stem straightness and vigour can also be deployed, unlike clonal seed orchards, where progeny trials are needed for that purpose. The following bullet addresses that point.

⁴See <https://www.sciencedirect.com/science/article/abs/pii/S0022191015000992>

- The application of forward selection in BSO versus backward selection in CSO? Is it relevant to use 'omic tools to support selection?

Comment: Forward selection and BSOs are well suited to species such as ash and birch which flower early (compared with oak and other long-lived species) and profusely in their life cycle. Clonal seed orchards are sometimes preferred in species such as oak where the use of physiologically older scion wood can bring forward seed production earlier than in seedlings. However, resort must be had to progeny to estimate heritability and for recurrent selection. A further consideration in the case of ash is the need to respond to a threat to the viability of the species at a landscape level and there the use of forward selection can lead to more rapid determination of heritability of ADB tolerance, and at the same deployment of tolerant material.

- Is the predicted 39% healthy trees at age 5 sufficient to form a viable ash forest?

Comment: Due to problems in the orchard's establishment, it is not certain that this is the level of survival one would get from seedlings from the final 350 trees. But this relates to the discussion under 3.2 and the level of survival from BSOs. Under current grant aid conditions, a survival of 35% as documented for the 2nd generation orchard would not be acceptable for pure ash establishment, but as a component of mixed stands it may be acceptable. Mixtures also have a dilution effect on ADB spore production, and this is likely to reduce disease incidence and severity (See Conference report). The point here is the same as 3.2 – a need for field trials and demonstration areas focussed on mixtures and deployment of tolerant trees and gene flow to the landscape.

- Use of the low input, high diversity BSO concept in ash breeding? Other strategies?

Comment: See earlier comments. The approach here is more along the CSO line. It has the advantage of being more accommodating to intermittent effort and perhaps with higher genetic gain initially. Both approaches can also proceed in parallel. Thought needs to go into the best strategy to adopt for Ireland. In that regard ongoing and close collaboration with leading parties in ash breeding and deployment in Denmark, Austria and UK and with emerald ash borer work is essential

Session 1 Resistance and Breeding

Richard Sniezco (US Forest Service), invited speaker 'Forest tree disease resistance breeding- successes and lessons learned'

The starting principle is that some level of resistance can be found within the vast gene pools of trees so it can be harnessed and developed by breeders. The breeders' principles are to develop populations of trees with genetic resistance that is at a useful level and frequency as well as being durable and stable while also maintaining genetic diversity and adaptability within the species. US programmes in developing resistance **to Emerald Ash Borer and to ash dieback are at the early stages and the key elements for success in breeding programmes include having breeding expertise, research, infrastructure, co-operators, management, continuity of funding and public support.**

From the ongoing US programmes, seeds are now available and are being utilised in reforestation and restoration for resistance to: white pine blister rust (*Cronartium ribicola*). *Phytophthora lateralis* in *Chamaecyparis lawsoniana* and *Fusarium oxysporum* in *Acacia koa*.

He emphasised the need to define 'success' in breeding for resistance in a given species. Durable resistance is required over many years and even decades and the stability of resistance must be demonstrated in many diverse environments. Expectations on the level of resistance need to be defined; it may be 100% for street trees whereas it may be as low as 20% survival if resistant trees are deployed in mixed forests where natural processes would reduce the population to this level. He also reviewed single gene and polygenic resistance as well as the constraints on seed production. He emphasised that molecular biology approaches are support tools but cautioned on tendencies of allowing molecular biology to dominate the main action in breeding programmes.

Dheeraj Rathore et al., Teagasc Forestry Department, Ireland *Ash Dieback Research and Breeding Efforts in Ireland*

Ash dieback, caused by the fungal pathogen *Hymenoscyphus fraxineus*, has led to widespread decline of common ash (*Fraxinus excelsior*) in Ireland since its detection in 2012. Research initially focussed on identifying healthy trees in the Irish population at a time when dieback disease was not very severe. Ten ramets of each of 100 Irish genotypes were therefore screened in Lithuania and 35 of these with lower susceptibility were repatriated to Ireland for further screening. Thereafter an additional 165 genotypes have been selected

and field planted for screening at two sites Castlemorris and Phoenix park. Resistance was scored in four categories and it varied with the site.

Research has evolved into coordinated breeding and restoration strategies, integrating field phenotyping, pathogen studies, molecular approaches, propagation systems, and silvicultural research. The work is led by Teagasc and involves partnerships with the Department of Agriculture, Food and the Marine (DAFM), Agri-Food and Biosciences Institute (AFBI), University College Dublin (UCD), and other national and international partners.

This major initiative, AshforFuture funded by DAFM and DAERA-NI, aims to identify, conserve, and propagate ash genotypes with reduced disease severity. Activities include field surveys, stakeholder engagement, and citizen science to collect tolerant ash trees across Ireland and Northern Ireland. Selected material is used in conservation collections, grafting, propagation, and new field trials to develop future breeding populations.

Additional research activities include studies on pathogen virulence and molecular responses linked to tolerance, the use of remote sensing and disease monitoring technologies and virome⁵ analysis and development of micropropagation systems for tolerant ash material. A specific study on ash viruses in Ireland, confirmed the presence of two which are common in Europe and one other, potentially a nepovirus⁶ in two symptomatic trees. The mode of transmission is unknown.

Jasmin Barl et al. Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFP) Vienna, Austria. *Results from Austria's ash conservation programme: selecting ADB-resistant *Fraxinus excelsior* genotypes based on artificial inoculation with *Hymenoscyphus fraxineus**

ADB appeared in Austria in 2005. R&D has involved screening 35,700 progeny trees for tolerance to ADB which were derived from 685 putatively tolerant mother trees. Stem infection damage among the progeny was assessed over three years using a seven-point scale and the most tolerant phenotypes were selected. This work has resulted in the establishment of three seed orchards using tolerant material that is locally adapted.

Inoculating ash trees with varying degrees of pre-existing damage, confirmed that the resulting wood discolouration correlates significantly with the damage classes that were assessed previously, indicating that a visual assessment of tree health status was a

⁵ A virome is the complete collection of viruses found in a specific organism, ecosystem, or environment.

⁶ A nepovirus is a nematode-transmitted polyhedral (isometric) virus.

sufficient method to evaluate susceptibility. Furthermore, for field-resistant trees that were artificially inoculated, the length of wood discolouration ranged from 0 to 43 cm. This indicated a varying defence response; some trees were able to actively limit the spread of infection in wood while others succumbed but could withstand natural infection.

Jo Clark et al., Future Trees Trust UK *The Living Ash Project*

The Living Ash Project, started in 2013, aims to identify ash trees with high resistance to ash dieback. Over a thousand putatively resistant trees have been already selected over that time period, based on their resistance phenotype in areas with high infection rates. Initial screenings involved planting 155,000 saplings on 14 sites of which over 80% had died after 11 years. This was followed by screening 40,000 trees from which 412 were selected and grafted. Observations in the RAP trial consisting of over 30 European provenances from 2015 indicate that infection severity tails off and some trees showed lesion healing. Two clonal archives (planted 2 X 2 m) have been established: one in southern England (planted in 2019) and another in Scotland (planted in 2024). The more remote archive in Scotland serves as a safeguard against the potential arrival of the emerald ash borer. The ultimate goal is to identify the most promising individuals for a controlled breeding programme to ensure the future of ash trees in the UK.

Michelle Cleary Swedish University of Agricultural Sciences. *From resistance to restoration: the next challenges for European ash recovery in Sweden*⁷

The history of dieback research in Sweden since its first appearance in 2001 was reviewed. She discussed the issues involved in gaining acceptance by the forest stakeholders for tolerant material when it becomes widely available. Such issues include: providing evidence of durable resistance over time, availability of tolerant saplings from nurseries, acceptable growth rates on suitable sites and an estimate of the financial risks involved in using the tolerant material, i.e. the interaction of elements in ecology, technology and society.

Valentin Queloz Swiss Federal Research Institute *Influence of tree species composition and host density on ash dieback severity in an ash rich forest reserve in Switzerland*

A study in Switzerland showed that only 22% of trees were alive in 2026 (168 trees) out of 758 monitored from the beginning of the study in 2017, in a natural forest. Yearly mortality increased as basal area increased. Mortality varied greatly between studied plots from 90% in dense stands to 25% in mixed stands. In 2017 38% of infected trees had fallen over while

⁷ See <https://link.springer.com/article/10.1007/s13280-026-02467-y> for further information on ash tree selection and breeding in Sweden.

in 2026 90% had fallen over. Species diversity also affected mortality rate. Locations with more sycamore had less dieback mortality.

William O'Neill et al., AgriFood and Biosciences Institute Belfast N. Ireland *AshForFuture: Harnessing natural variation in Fraxinus excelsior for ash dieback tolerance in the Irish landscape.*

This is a collaborative project (Teagasc, AFBI, UCO, FGRT) aiming to: Identify ash trees with potential tolerance to ash dieback. Propagate these tolerant genotypes. Establish a living gene bank for conservation and breeding. Grafting material was collected from 18 candidate trees (17 from County Kilkenny, 1 from County Mayo). Selection was based on a 90% crown fullness threshold in a 30-year-old, ash-dominated stand. Healthy trees represented only 0.3–0.5% of the assessed population. In April 2026, scions from 11 candidates showed some ash dieback symptoms; 6 remained symptom-free, highlighting the need for further validation. Scions were grafted onto ash rootstocks and will be used for further screening and establishing a genebank.

The project will expand into surveys across 45 infected ash stands in Northern Ireland during 2026. The project has already identified and propagated candidate tolerant trees, and is setting up infrastructure (gene bank, scoring systems) for ongoing selection and breeding. Standardized scoring and permanent identifiers will enable long-term tracking and comparison.

Katharine Budde and Gitta Langer Northwest German Forest Research Institute Hann Munden and Gottingen Germany *Applied ash dieback research in Germany*

Roughly one third of all ash trees in Germany have died from ADB and local mortality rates exceed 80%. Studies are underway to develop management and mitigation strategies in the forest. Out of 14,000 trees evaluated, 200 were selected for grafting and subjected to natural ascospore infection. From these 96 tolerant trees were selected and used to establish four clonal seed orchards. In reciprocal transplant experiments it was shown that transplanting saplings from severely affected sites continued to be more healthy than those transplanted from moderately affected sites. This prompted a study to determine if exposure to strains of *H. fraxineus* with a low virulence could prime the defences in susceptible trees.

Session 2 Genetics Genomics and prediction models

Joanna Meger et al., (Dept. Genetics Kazimierz Wielki Univ. Poland), **invited speaker**
*Genomic Resources for Ash Dieback Resistance and Functional Sex Identification in European Ash (*Fraxinus excelsior*)*

Whole-genome sequencing was performed on 320 ash trees from across Poland. Over 26 million filtered SNPs (single nucleotide polymorphisms) were analysed for associations with disease resistance and functional sex expression.

A total of 414 highly significant SNPs were associated with resistance to ash dieback, with more than 51% located on chromosome 2. A notable cluster of 158 SNPs was found in a 15 kb region on chromosome 2, suggesting a key candidate region for resistance genes. High SNP-based heritability estimates confirm a strong genetic component to disease resistance. Genomic prediction using a 500-SNP panel achieved high accuracy ($r = 0.89$ in internal validation, $r = 0.80$ in external validation), supporting its use in breeding programs.

Ash trees may be functionally male female or both sexes, they exhibit a mixed sexual system (trioecy or subdioecy⁸), making early sex identification important for breeding and seed orchard design. 3,884 significant SNPs were associated with functional sex, with over 68% on chromosome 6. A candidate sex-determining region of about 1.73 Mb on chromosome 6 was identified, containing 2,370 significant SNPs.

Dario Galanti et al., RBG Kew and Queen Mary Univ London *Genetic Variation and Structural Variation*

The role of genetic and structural variation in the adaptability of *Fraxinus excelsior* (European ash) to ash dieback disease (caused by *Hymenoscyphus fraxineus*) was investigated. Researchers sequenced approximately 950 trees from three UK populations, enabling parent-child relationship assignments and genome assembly. Extensive genetic variation, including structural variants (SVs), was detected and genotyped across samples.

⁸ Trioecy is a system where males, females, and true hermaphrodites coexist as three distinct, stable sexual classes, roughly split into pure males, pure females, and perfect cosexuals. Subdioecy is a system that is mostly dioecious (separate male and female plants) but includes a few individuals or hermaphrodites, that express the opposite sex weakly.

Genome-Wide Association Studies (GWAS) confirmed that susceptibility to ash dieback has a polygenic architecture, and that not accounting for SVs can inflate association signals. Large-effect regions, such as those determining self-incompatibility groups, were identified as important sources of genetic variation for disease resilience.

Elizabeth Orton et al. John Innes Centre Norwich UK. *Heritability resistance and tolerance to ash dieback*

Field studies in coppiced trees, estimated broad-sense heritability of resistance (73%) and tolerance (52%) to ash dieback indicating a strong genetic component. Heritability was highest in woods with moderate disease severity and high variation between trees. Visual selection of healthiest trees is a reliable method for choosing breeding stock. Inoculation tests on half-sib progeny showed high heritability for lesion size in both rachis (78% for family means) and stem (67% for family means), supporting the use of these tests for identifying resistant parental lines. There is a high correlation (0.69) between rachis and stem lesion sizes, further validating inoculation tests for breeding purposes.

Phoebe Swift et al., Royal Botanic Gardens Kew and Queen Mary Univ. London *Genomic Basis of Ash Health After Long-Term Exposure to Ash Dieback*

Resilience to ADB is polygenic (involving many genes) and heritable, suggesting that evolutionary rescue of the species is possible. Maintaining genetic diversity is crucial to protect against future threats, such as the emerald ash borer (EAB).

Whole genome sequencing (WGS) was performed on 2,750 UK ash trees to study health and survival under ADB. A K-mer based genome-wide association study (GWAS) was performed and identified novel structural genetic variants linked to tree health and survival from 1500 living trees and 780 which had died. The distribution of these variants was analysed, with the hypothesis that survival-associated variants would be more common in younger (juvenile) trees.

Health, survival-, and genome-wide K-mers were used to build a Genomic Prediction (GP) model to estimate an individual tree's genetic merit for survival based on genotype data. The GP model will be validated using mature trees to ensure predictions align with observed health outcomes. The validated GP model can be used in breeding programs to select young trees with high genetic potential for survival, accelerating the process by removing the need to wait decades for phenotypic data.

Mari Mette Tollesfrud et al., (Norwegian institute of Bioeconomy Research As Norway). *Assessing genomic relatedness and the potential for in situ heritability estimation in natural populations of European ash*

The study aims to assess in-situ heritability of ash dieback tolerance in natural forest populations, which is crucial for understanding the evolutionary potential of ash and informing forest management and conservation strategies.

Sampling was conducted in two heavily diseased ash forests (one in Denmark, one in Norway). Both adult and juvenile ash trees were sampled within regeneration plots. Both groups were assessed for ash dieback symptoms; juveniles were also evaluated for phenology and height growth over three years. Around 600 individuals were genotyped using the 4TREE SNP array.

In six research plots 80 adults and 240 juveniles (320 genotypes) were phenotyped and genotyped. Disease damage was assessed using four categories Zero for fully healthy and a Four for a dead tree. Results showed that Danish populations showed a greater genetic relatedness between adults and juveniles than Norwegian populations. Heritability values for bud flushing were higher in Danish material compared to Norwegian over three successive years.

Ongoing analyses include genomic relatedness, parentage structure, and estimation of additive genetic variation under natural conditions. Results will provide insights into whether genetic variation in tolerance persists in natural settings, with important implications for conservation, forest management, and enrichment planting.

Jill Hamilton et al., (Penn State Univ USA) *Evolution on two continents, genetic structure and diversity of green ash (*F. pennsylvanica*) within its native and introduced range*

This study compared the genetic structure and diversity of Green Ash (*Fraxinus pennsylvanica*) across its native North American range and its introduced European range (Germany and Austria). The research used whole-genome nuclear and chloroplast sequence data from 300 individuals (25 North American populations, 18 European populations). This research showed that Austria has two chloroplast types while USA has four in *F. pennsylvanica*. The Austrian population has diverged from the USA population. In the USA there is a clinal latitudinal variation with *F. americana* replacing *F. pennsylvanica* in

a southerly direction. Hybrid populations were found in Austria and they may have been introduced from USA.

Alberto Santini et al., *Deeply divergent evolutionary lineages and clonal dynamics of *Hymenoscyphus fraxineus* in Italy: insights from whole genome sequencing*

Hymenoscyphus fraxineus, in Europe originated from a severe genetic bottleneck involving just two divergent founder strains from Asia. The main aim was to characterize the population structure of *H. fraxineus* in Italy. For this, 92 haploid isolates were analysed (72 from Italy, others from Sweden, Austria, Czech Republic). Whole-genome sequencing (WGS) using Illumina technology, yielding about 4.23 billion reads. Data filtered and analysed using PCA, ADMIXTURE, and Gaussian Mixture Models on pairwise genetic distances.

This research identified the presence of three primary genetic clusters in Italy. The clusters show "fixed" ancestry with negligible recent gene flow, even in geographically close populations. Genetic distances are bimodal: low within clusters (median 0.02), much higher between clusters (median 0.30), with statistical tests confirming clear separation. The Monte Tavianella Bacca di Rio site in Emilia-Romagna is a notable area where two distinct lineages coexist without recent mixing (sympatry). The presence of independent evolutionary units suggests pathogen diversity is maintained by multiple introductions. This has major consequences for monitoring, management, and breeding for resistance, as new introductions from Asia could increase the pathogen's adaptability and disease severity.

Session 3 Biodiversity Ecology and Restoration

Jurgen Bauhus Univ Freiburg Germany *Can European ash forests be restored*

We need to consider our ideas of 'Restoration' in relation to ash and the material which is coming from breeding programmes. The concept of 'prestation' involves preparing for novel future conditions by transforming forest composition and function. Combining multiple restoration approaches increases resilience and options for uncertain futures. Engage stakeholders in decision-making and communicate transparently about risks and benefits.

Restoration strategies must consider two scenarios:

(a) The discovery and deployment of resistant/tolerant ash genotypes and containment of pests such as EAB. If resistant or tolerant ash genotypes are found and pest spread is contained, restoration can focus on propagating these genotypes. Initially, the supply of resistant genetic material will be limited, so efficient use (e.g., cluster plantings) is recommended to maintain 'ash continuity.'

(b) Continued spread and devastation by these pests. If pests continue to devastate ash populations, restoration must shift to maintaining ecosystem functions and biodiversity.

These strategies must include preserving genetic diversity in existing ash populations., maintaining ash legacies (dead wood, associated fungi, lichens, insects), and introducing other tree species with similar ecological functions as *F. excelsior*. Developing admixtures which can maintain ash - associated species diversity by deploying oak, alder, *Ulmus laevis*, *Prunus* spp and concentrate planting on sites with low disease severity. While buying time for resistant material becoming widely available it is essential to encourage natural regeneration, retain and maintain healthy trees within heavily diseased stands, especially seed-bearing trees and establish refugia sites. The use of Asian ash species or hybrids (which have co-evolved with these pests), such as, *F. mandshurica*, *F. chinensis* and *F. platypoda* may be considered, but requires careful risk assessment.

F. H Schei and M. M Tollesfrud Norwegian institute of Bioeconomy Research Norway *A keystone in decline- Ash dieback consequences in western Norway*

Ash comprises only 1% of the forest in Norway mostly along coastal areas in association with elm (*Ulmus glabra*) lime and hazel. Dieback symptoms were found in 87% of trees.

Ground surveys of ash woodland ground flora from 1989 to 2007 were compared with current data on species composition in 82 plots on 23 sites. So far there has been no significant change in ground flora composition, but this may change as disease severity increases. Pollarded ash are an important cultural element in Norwegian landscapes and the act of pollarding is grant aided. However, pollarding appears to accelerate the death of trees and the grant policy may need to be revised.

Session 4 Future Threats- Emerald Ash Borer *Agrilus planipennis* (EAB)

Leigh Greenwood The Nature Conservancy Montana USA **Invited Speaker** *Lessons learned from 20 years of EAB resistance research in the United States*

EAB is an invasive pest in North America, threatening the survival of all native ash species (*Fraxinus*), especially white ash (*F. americana*), green ash (*F. pennsylvanica*), and black ash (*F. nigra*).

Within five years of detection a very clear decline and death of trees is evident. However, trees are typically infected for 3-5 years before adults EAB are detected. Pheromone trapping in the USA appear to have been ineffective as a tool for early detection of insects because while one tree may provide negative evidence of EAB, a nearby tree may be infested and remain undetected. Insect-infested trees may display symptoms which are typical of ash dieback. Once established the insect never disappears from an area and can persist even in stump sprouts from dying trees.

Insect spread is by transport of logs containing larvae. In the USA this is common. However, outbreaks have also been found in many places which are hundreds of kilometres from the nearest outbreaks, indicating long distance transport on communication routes on trains and highways. Adults travel on vehicles to destinations many kilometres from the original outbreak areas.

Larvae eat the phloem tissue under the bark and this eventually kills the trees. *H. fraxineus* is confined mainly to phloem and so the decline of trees is similar for both pests. After moulting and pupating under the bark, the adults emerge leaving a typical 'D' shaped hole and fly locally to infect other trees by laying eggs. The small larvae burrow into the tree.

In forests heavily impacted by EAB, some mature ash trees, called "lingering ash," have survived and maintained healthy canopies after peak infestation i.e. typically two years after mortality has levelled off. Many lingering ash trees show quantifiable resistance to EAB, which can be enhanced through breeding. Typically, the USDA selects one lingering ash tree in 10,000 trees for breeding.

Black ash, closely related to European ash (*F. excelsior*), shows a higher frequency of a specific resistance trait (proportion of EAB larvae killed) compared to green and white ash. Trials indicate that trees of European ash species (*F. excelsior* and *F. angustifolia*) have higher survival rates relative to green ash when exposed to EAB. There are likely enough EAB-resistant genotypes among European ash for structured resistance breeding programs to succeed in conservation and restoration. For Europe, the breeding goal must be to develop resilient ash populations with dual resistance to EAB and Ash Dieback.

Anastasis Uglov Forest Research UK *Host responses and potential diagnostic tools for Emerald Ash Borer in European Ash*

EAB is an invasive pest threatening ash populations in Europe and the UK. Detection is difficult because visible symptoms usually appear only when trees are already in severe decline. The study investigated early diagnostic indicators and host responses to Emerald Ash Borer (EAB) infestation in (*Fraxinus excelsior*) under UK conditions and also the interaction of ash dieback on tree susceptibility to EAB.

Trees of different ages and cut logs were inoculated with EAB eggs in controlled conditions and tissues were assessed after visible lesion formation or fluid exudation. Most inoculated trees showed purple discolouration (identified as cyanidin glucoside) of phloem tissue, which was linked to reduced number of EAB larvae developing to the adult stage. *F. excelsior* appears to suppress some stages of larval development, however it does not completely suppress the final emergence of all adults.

The presence and intensity of the purple zones were influenced by tree age; older trees had larger and more pronounced purple zones. The discoloration may reflect host defensive reaction or a physiological reaction to larval feeding. Fungal growth was notably reduced within purple zone areas. Phloem discolouration, lesion formation, and lesion-associated microbial communities may serve as early diagnostic indicators of EAB infestation.

Donnie Peterson et al., Swedish Univ Agricultural Science Alnarp Sweden *Interactive effects of the emerald ash borer (*Agrilus planipennis*) and ash dieback pathogen (*Hymenoscyphus fraxineus*) on volatile profiles and larval performance in three European *Fraxinus* species.*

This study highlights the interactions between the emerald ash borer (EAB) and ash dieback pathogen (*Hymenoscyphus fraxineus*) on European ash species. The combined effects on tree chemistry and insect performance are not well understood. The researchers tested three European ash species (*Fraxinus excelsior*, *F. angustifolia*, *F. ornus*) under four treatments: control, pathogen only, EAB only, and both stressors together. They measured

EAB larval development, fungal lesion length, and volatile organic compound (VOC) emissions.

Larvae of EAB developed significantly faster in *F. angustifolia* co-infected with *H. fraxineus* than in trees infested with EAB alone. Ash dieback infected trees increased larval growth in *F. angustifolia* but not in *F. excelsior*. *F. ornus* showed a marginal increase in larval development under co-infection, while *F. excelsior* showed no difference between treatments. Fungal lesion length was not affected by EAB presence.

In *F. angustifolia*, total volatile emissions (VOC) did not differ among treatments, but qualitative differences in VOC profiles were observed in *F. excelsior* and *F. ornus*. Key compounds distinguishing treatments included green-leaf volatiles (3-hexen-1-ol, 3-hexenyl acetate, 3-hexenyl butyrate) and methyl salicylate, which are known to attract EAB. Infection by *H. fraxineus* can enhance EAB larval growth in *F. angustifolia*, indicating species-specific responses to combined biotic stress

This study suggests that for *F. angustifolia* populations, simultaneous outbreaks of both EAB and ash dieback could lead to faster tree decline due to increased EAB larval performance. It may also suggest that *F. angustifolia* could act as a potential sentinel species for early detection of EAB although *F. pennsylvanica* would be the most appropriate lure due to its severe susceptibility to EAB.

Rimvys Vasaitis et al., Swedish University of Agricultural Science Sweden *Invasion of Emerald Ash Borer and Ash Dieback in Ukraine – concerted Action: an update year 2025*

EAB (*Agrilus planipennis*) was first detected in eastern Ukraine in 2019. The beetle initially infested American ash (*Fraxinus pennsylvanica*) and then spread to native European ash (*F. excelsior*) in both forests and urban areas. The EAB invasion is accelerating, with more trees infested in previously affected areas and expansion into new territories. It has been confirmed in Slovakia and Hungary this year.

Although the natural rate of expansion of EAB infestation is 10 km / year, in 2022, EAB was found in Kyiv, over 500 km from the original eastern population, likely due to "hitchhiking" on transport vehicles. *F. excelsior* is more susceptible to ADB than *F. pennsylvanica*. The combination of EAB infestation and ADB infection is expected to be more lethal than either threat alone. At early stages, ADB infection may predispose *F. excelsior* to EAB attack. At later stages, EAB increasingly attacks and kills even healthy trees, regardless of ADB symptoms. It is very difficult to detect the initial infections visually. Removal of infested trees in which typical exit holes are visible is too late to halt expansion of this pest.

This ongoing study is to investigate the expansion range of EAB in Ukraine and assess the relative susceptibility of *F. excelsior* and *F. pennsylvanica* to EAB and ADB. Study the combined effects of both pests on ash tree health. Ongoing research is to identify and map potentially resistant or tolerant European ash trees (*F. excelsior*). For monitoring we use of green and violet canopy traps in both random and targeted locations and also testing the efficacy of traps.

Field trips (please refer also to the handouts for each day attached with the report)

1. Background

Selection and breeding of ash in Sweden and Denmark began well before ash dieback (ADB) was first detected in 2001 and 2002, respectively. Ash breeding activities in Denmark date to 1934 when the first plus trees were selected. With the advent of scientific tree breeding ash was the first tree species targeted with the establishment of first clonal seed orchard in 1947. The first plus tree selected also turned out to confer a high level of ADB on its progeny. The existence of ash seed orchards prior to the arrival of ADB, allied to scientific capacity, allowed Denmark to respond to ADB through early detection and the design and establishment of seed orchards, with some now in the second generation.

2. South Sweden, 26 August 2026

Selection of ash by Skogsforsk (the Swedish forest research agency) and partners began in south Sweden in 1991 when 106 plus trees were selected in 17 stands. These were grafted and planted randomly in two seed orchards, at Snogeholm in 1992 (50 ramets) and at Trolleholm in 1995 (10 ramets). Snogeholm was the site visited. Following the detection of ADB in 2001⁹ its incidence was first scored in 2006 using a system that runs from 1: no infection to 9: dead. A further six annual assessments were made, with the most recent in 2026. At Snogeholm there has been 90% mortality, with just over 2% of trees scored as having no infection, which is at or above observations in plantations and stands in Ireland and the UK. The best clones performed consistently across both sites, indicative of a lack of genotype x site interaction. It is notable that in 2026, 71 trees produced seeds among 31 genotypes. The trials continue to be monitored. Perhaps surprisingly the fencing has not been maintained at Snogeholm, and there has been some browsing damage. There was no reason given for the fencing not being maintained.

Since 2014, mature ash that remained healthy in heavily affected stands (> 80% crown vitality) have been continuously selected across Sweden. Starting in 2015, scions were grafted onto European ash rootstocks (mixed sources) and planted in three separate clonal

⁹ See Stener, L.G., 2018. Genetic evaluation of damage caused by ash dieback with emphasis on selection stability over time. *Forest Ecology and Management*, 409, pp.584-592.

trials at Snogeholm the following year. Each trial contains different clones; performance and genetic parameters were therefore analysed separately within each trial. The small size of trials reflects the limitations (funding, available rootstock).

The trials established at Snogeholm in 2016, 2017 and 2018 were

2016: 65 clones European ash, including some seed orchard selections + Radda Asken¹⁰ selections, susceptible controls (4), and Asian references (5) – up to 13 ramets/clone

2017: 54 selected European ash clones- 7-13 ramets/clone

2018: 36 selected European ash clones – 7-13 ramets/clone.

The Asian ash¹¹ material performed best regarding ADB tolerance in the 2016 trial (see pdf of handout) with 3 clones on average with over 70% of the trees symptomless. Three of the seed orchard clones (presumably tolerant material from the 1991 / 92 trials) were among the top 10 performers, with the remainder of the top 10 being among the subsequently selected ash trees.

3. Denmark 27 August 2026

Stop 1:

Scion trial Horsholm - study site for quantitative genetics studies on progenies, studies on parent offspring correlations in ADB susceptibility and inoculum source for other studies.

1.1 *Quantitative genetics of progeny from clonal seed orchards and ash populations*

The trial was planted in 2001 and consists of (a) putative half-sib offspring from three forests allowing estimation of genetic parameters and (b) from three seed orchards and second generation from the same seed orchards based on seed collected in plantations which originate from the clonal seed orchards.

¹⁰ Radda Asken (Swedish for "Save the Ash") is a citizen science and research initiative in Sweden launched in 2013 to protect and conserve the European ash tree (*Fraxinus excelsior*) from extinction. <https://link.springer.com/article/10.1007/s13280-026-02467-y>

¹¹ Species were *F. platypoda*, *F. mandshurica*, *F. japonica*, *F. spaethiana*

Key results: significant levels of additive genetic variation with moderate to high narrow sense heritability. Large differences were found in offspring survival between seed orchards. For example, survival at age 11 in offspring from the FP22 seed orchard was about 70% for both 1st and 2nd generation material, but much lower in FP212 and FP217 offspring. The survival differences may be due to ADB (but bearing in mind that ADB was first detected in DK in 2002), since the seed orchards include different clones.

The offspring from FB217 have in general grown slower than the offspring from the two other seed orchards. The growth rate of 1st generation offspring collected in FP202 was significantly higher (on average 20%) than corresponding second generation offspring. This could indicate inbreeding depression. However, it was not the case for one first and second generation from FP217 (where the highest effect of inbreeding depression would have been expected). A further possibility to consider is that SSR based analysis of trees in the scion trials showed that the seed orchards were poorly isolated from pollen flow (e.g. 50% pollination into seed orchard FP 212), and in the second-generation stands is more likely. The differences between 1st and 2nd generation may therefore be due to different pollen clouds rather than inbreeding.

These findings point to the need to carefully monitor the performance of seed stand progeny, for both ADB tolerance and growth performance, and the need for caution in recurrent selection to avoid inbreeding depression leading to loss of vigour and a reduction in overall genetic diversity. This is in fact a widespread concern across tree breeding programmes, but there are well understood ways to test for and avoid such effects.

1.2 Parent-offspring correlations in ADB susceptibility

A short presentation was given on results of artificial inoculation of ash stems using *H. fraxineus* infected wooden plugs, across 125 progeny from 5 mother genotypes from trials. Infection severity was assessed by visual assay of the length of necrosis adjacent to the plugs. This was compared to the average crown damage across the 5 genotypes. There was a close correlation ($r = 0.75$) between crown damage and average necrosis across the genotypes. The utility of induced necrosis development for detection of ADB tolerance in younger trees and progeny as opposed to crown damage score is however open to question regarding mature trees especially. Field survival and vigour are the ultimate test of ADB tolerance, as tested in gene banks and progeny trials. Infection studies may offer early screening but as a guide to progeny selection their utility remains to be fully demonstrated over the full life of trees.

2.1 Species variation in ADB susceptibility

The arboretum at Horsholm contains 17 *Fraxinus* species from Asia and North America, with most represented by several species. These specimens were used to explore variation in ADB susceptibility across species by observations of disease symptoms in the arboretum, stem inoculation of grafted plants and spore infection of leaves. Species in clade *Fraxinus* accumulated high levels of *H. fraxineus* in their leaves, but the non-Asian ones had higher levels of induced necrosis (see Figure 2.1 in handout).

Further exploration of the use of controlled crosses with *F. mandschurica* merits consideration in Ireland in the context of ADB and the threat from emerald ash borer which is progressing across Europe (see conference report).

2.2 *In situ* conservation gene conservation of ash

In situ conservation of ash stands began in Denmark in 1993 based on evolutionary conservation. The work is now part of EUFORGEN collaboration and involves visiting conservation stands of ash and monitoring of forest dynamics and assessment of the level of genetic diversity using SNP assays. Contact emails for the persons involved in the work are provided in the attached handout.

As to the efficacy of *in situ* conservation this interacts with current DAFM measures to remove diseased ash stands and replacement with another species. FGRT has advocated for asking landowners to retain healthy ash when removing diseased stands. This may be difficult to implement administratively but is desirable to retain genetic diversity in the form of residual trees which may represent less than 1% of the total population. Furthermore, residual healthy trees may produce seeds which can scatter locally and which contain a proportion of progeny with improved tolerance to ADB. Similarly, retained trees may produce pollen carrying genes which may confer tolerance on progeny when they fertilise other healthy trees in local environments. FGRTs work, allied to the AshForFuture project provides for a level of *ex situ* conservation, in particular our planned gene banks at JFK and Loughgall. There may however be merit in designating areas for *in situ* conservation of genetic diversity in NPWS owned areas. These may serve as resources for ongoing screening of selected material and would have potential to be converted to either seed orchards or seed production areas after rogueing of susceptible trees.

3.1 *Policy importance of ash and seed supply*

Prior the advent of ADB in 2002-2003 ash was a minor species in breeding and seed procurement Denmark. The response to ADB across *in situ* conservation, selection and breeding activity over the intervening two decades is evidence of its current importance as a species. The work over the period has resulted in novel approaches to selection and the beginnings of deployment of tolerant material to forest planting. Meanwhile seed orchards are now in the second generation.

Deployment of tolerant material requires careful consideration. In the Ireland context a way forward is through field trials/demonstration areas with Danish seed orchard material planted pure and in mixture. An important consideration is that seed orchard material that is currently available will not provide for survival rates in excess 80-90% which is an expectation in forest establishment. Nevertheless, lower survival rates may be sufficient for silvicultural purposes, particularly in mixed stands. This topic was specifically addressed at stop 3.2.

3.2 *Stand dynamics in mixed ash stands*

Research has shown that ash seed and especially pollen can disperse for up to a kilometre from the parent tree, promoting gene flow into the surrounding landscape (this was part of the FGRT argument for asking owners to retain healthy trees when felling ash areas). A new project called CAPSIS is underway which will evaluate alternative management strategies for ash. A working hypothesis is that for long rotations of ash, say up to 80 years, cumulative volume production in stands with as low as 20% of full stocking will not be significantly different from stands with full stocking. This accords with first principles of stand growth, and with silvicultural practice where the final stocking in broadleaf management is less than 20% of the stocking at establishment/regeneration. This points to the need to consider the deployment issue, and related rotation lengths and silvicultural systems to promote gene flow from tolerant trees, including the management and thinning of stands for wood production.

This stop raised the question as to options for diseased stands and how they should be managed. One option is enrichment planting using tolerant material. This is feasible in mixed species stands in Europe. However, based on practice in Ireland and owner considerations complete replacement of the stands seems to be most practical and acceptable option.

However as more tolerant material becomes available from Danish and Irish sources options such as enrichment and conversion to continuous cover systems will become available.

3.4 Development of mortality in ash stands

This work involves ADB monitoring in naturally regenerated ash in small fenced off areas under a mixed species forest canopy. It is known that healthy ash trees have higher reproductive success than heavily diseased trees. It is also known the heritability of ADB tolerance is moderately high. The hypothesis is therefore that with natural regeneration tolerant trees will come through and better adapted ash will emerge through natural selection. In sites with natural regeneration and healthy mature trees, the cohorts of regenerants were genotyped and assigned to both fathers and mothers within the site. The trial is at an early stage and early estimates show a higher heritability for bud burst than for crown damage, with segregation of trees by ADB symptoms and mortality just emerging.

Work of this nature is important as instances of prolific ash regeneration can be observed in stands impacted by ADB. Work on this topic is also underway in GB, with the question can natural regeneration provide sufficient trees to occupy the site and function as forest. FGRT observations have seen instances of extremely high seedling mortality, due to ADB, once a height of 1-2 m is reached. Nevertheless, it is an area where collaboration with work in GB and Denmark might be considered.

4.1 Tjaereby multi-generational breeding seedling seed orchard (BSO)

This is second generation BSO orchard planted in 2022, comprising 3483 progeny from 77 open pollinated families collected from a first generation BSO in Randers Fælled, where seed were collected at age 14 from healthy trees (about 4% of the breeding population). Following establishment there were losses due to drought, then waterlogging and spring frost in 2025. Current survival is 39%. Seed from the orchard will likely become available early in the coming decade.

The handout posed a number of important questions, not all of which were discussed during the visit:

- Selection was designed to keep high effective population number - would it have been relevant to select stronger within and between families to get higher gains?

Comment: This is one of the most common considerations in tree breeding programmes. The Danish approach is forward selection by seed collection from putatively tolerant parents in the forest, establishment of a BSO, rouging to remove diseased trees and reduce the

breeding population and provide space for crown development and seed production as the next bullet outlines.

- The plan is to continue strong forward selection down to 350 trees during the next 10 yrs to increase gain, allow selection for other criteria and ensure abundant seed collection.

Comment: Assuming plant spacing was close to 2x2 m for c 3500 trees, the final 350 trees will have a spacing of c 40 m², c 6x6 which seems adequate for seed production. With a BSO selection for traits such as stem straightness and vigour can also be deployed, unlike clonal seed orchards, where progeny trials are needed for that purpose. The following bullet addresses that point.

- The application of forward selection in BSO versus backward selection in CSO? Is it relevant to use 'omic tools to support selection?

Comment: Forward selection and BSOs are well suited to species such as ash and birch which flower early (compared with oak and other long-lived species) and profusely in their life cycle. Clonal seed orchards are sometimes preferred in species such as oak where the use of physiologically older scion wood can bring forward seed production earlier than in seedlings. However, resort must be had to progeny to estimate heritability and for recurrent selection. A further consideration in the case of ash is the need to respond to a threat to the viability of the species at a landscape level and there the use of forward selection can lead to more rapid determination of heritability of ADB tolerance, and at the same deployment of tolerant material.

- Is the predicted 39% healthy trees at age 5 sufficient to form a viable ash forest?

Comment: Due to problems in the orchard's establishment, it is not certain that this is the level of survival one would get from seedlings from the final 350 trees. But this relates to the discussion under 3.2 and the level of survival from BSOs. Under current grant aid conditions, a survival of 35% as documented for the 2nd generation orchard would not be acceptable for pure ash establishment, but as a component of mixed stands it may be acceptable. Mixtures also have a dilution effect on ADB spore production, and this is likely to reduce disease incidence and severity (See Conference report). The point here is the same as 3.2 – a need for field trials and demonstration areas focussed on mixtures and deployment of tolerant trees and gene flow to the landscape.

- Use of the low input, high diversity BSO concept in ash breeding? Other strategies?

Comment: See earlier comments. The approach here is more along the CSO line. It has the advantage of being more accommodating to intermittent effort and perhaps with higher genetic gain initially. Both approaches can also proceed in parallel. Thought needs go into the best strategy to adopt for Ireland. In that regard ongoing and close collaboration with leading parties in ash breeding and deployment in Denmark, Austria and UK and with emerald ash borer work is essential.

4.2 Genomics and epigenomics in ADB research

Comment: Refer to handout for details. Work confirmed that ADB tolerance as polygenic. Also, the utility of genomics in identifying regions of the genome related to tolerance is increasing and may have relevance to selection and breeding. Nevertheless, the scientific consensus among ash breeders is the need for the continuance of classic selection and BSO/CSO establishment allied to ex situ gene banks, to provide tolerant ash for deployment in silviculture and spread of tolerance in natural populations.

The link between early leaf senescence and reduced levels of crown damage has been proven in field trials and is supported by observations here. The exact mechanism is unclear. It may indicate that early senescence in leaves prevents the ingress of the fungus into the stems. This observation raises questions about the role of gene regulation (epigenetics) in mediating tolerance. Genomic studies indicate that tolerance to ADB is genetically based but the genes and gene combinations are not known. Coding regions outside genes and structural elements are a feature of the tolerance response of trees and this supports the gene regulation hypothesis. These are areas that merit further research.

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