

FraxNet meeting Nottingham University 29/11/2023

Introduction

This one-day meeting was organised under the auspices of EUPHRESKO by Dr E Orton to discuss collaboration in research in combatting Ash Dieback (ADB) and Emerald Ash Borer (EAB) in *Fraxinus*. Countries currently participating in EUPHRESKO include Austria, UK, Slovenia, Slovakia, Sweden and Ireland and it is open for other countries to join.

Themes of the EUPHRESKO action are:

1. Reviving ash populations
2. Future threats to ash such as EAB
3. Biodiversity and ecology implications.

Presentations

MICHELLE CLEARY (Swedish University of Agricultural Sciences, Sweden) The **Pathobiology of Ash Dieback: Exploiting Host Resistance to Ensure a Future for European Ash**

An overview of ADB in Europe was provided. Main points:

- The disease was recorded earliest in Lithuania, confirmed in 1997 and was present decades earlier.
- There is a long time-lag from first detection to severe disease.
- Threat to *Fraxinus excelsior* as a species in 2010 was categorised 'Threatened' and progressed to 'Critically Endangered' by 2015.
- Damage is greater on wet sites, and *Armillaria* accelerates mortality rates.
- Seeden has two clonal seed orchards planted in 1992 and 1995 with 10- 60 ramets of each of over 100 genotypes. They were assessed for ADB in 2006, 2007, 2010, 2011, 2016, 2021. Overall mortality was 89%.
- Clones selected which initially showed tolerance and susceptibility to ADB have remained stable in their respective health conditions from 2006.
- They have used public consultations to construct a data base for potentially tolerant trees which are routinely assessed, with material from such trees propagated: 65 in 2016; 65 in 2017 and 42 in 2018 for further screening.
- Research on developing a chemo typing of healthy and susceptible trees based on Ramen Spectroscopy is being developed. First results have shown clear differentiation signals for each phenotype.

RICHARD WHITTET **Latest results from ash dieback screening trials: Prospects for a resistance breeding programme** (Forest Research, Northern Research Station, UK and Future Trees Trust)

Information was provided on the results of a mass screening trial and of half sib progeny trials in relation to ADB. Main points:

- Mass screening trials were set up in 2012 using 155,000 ash trees on 14 sites, with six sites remain after consolidation. Two Irish provenances were included.
- Currently 20% of trees remain alive (80% mortality). No tree was totally resistant. Currently the level of tolerance was estimated as 1.5% representing 296 trees from trials.

- Early selection of individuals at 3-7 years was unreliable.
- In 2016 a further 3 sites were planted using half sib families to estimate genetic parameters across sites. Tolerance levels across sites were consistent regarding family responses.
- Site effects were strong and site management was challenging.
- It was suggested that re-selection of tolerant individuals from within the most tolerant families was an optimal way to proceed.
- The value of intensive phenotyping, top grafting, early flowering, genomic prediction and infusions of germplasm from elsewhere were discussed as options to consider in relation to breeding ash.

LAURA KELLY (Royal Botanic Gardens Kew, UK. Comparative genomics of *Fraxinus* identifies candidate genes for low susceptibility to ash dieback disease

Natural hosts of the pathogen *Hymenoscyphus fraxineus* (ADB) and of *Agilus planipennis* (EAB) may have resistance genes among ash species in Asia where these pests are endemic but cause no harm to trees.

- Research on EAB has identified genes for a defence response in these species by using a comparative genomic approach that indicated a comparative molecular evolution between phylogenetic lineages.
- A similar approach was adopted in relation to ADB in which 69 amino acids spanning 62 gene variants showed convergence between *Fraxinus* lineages with low susceptibility to ADB.
- 18 of these genes had a putative function in defence responses to fungi and 12 were linked to general defence mechanisms.
- A few other studies have identified the activation of defence genes but only three of the identified genes were in common across these studies
- These studies seem to indicate that the genomic basis for resistance to ADB in Asiatic spp such as *F. mandschurica* may be different to those operating in tolerant trees of *F. excelsior*.

DAEGAN INWARD (Forest Research, Alice Holt Lodge, UK) The resistance of UK treescapes to Emerald Ash Borer

In anticipation of the entry of Emerald Ash Borer (EAB) to UK work has been initiated to investigate resistance testing on *F. excelsior*.

- EAB is secondary pest on stressed ash trees in Asia. It has devastated ash in the US when it arrived, especially *F. pennsylvanica* which is now classified as ‘Critically Endangered’. It is now in 35 states, including in the latest Oregon, and has been identified in Kiev in 2023.
- The 2-year life cycle was outlined, eggs laid on bark externally after adult canopy feeding, pupation of larvae in sapwood and spring emergence of adults.
- Some observations suggest that *F. excelsior* is resistant to EAB but results are conflicting.
- Lab studies are underway on inoculating branch sets to study the effects of temperature and other factors on insect development to determine if European climatic conditions may limit or enhance the risk of potential EAB introduction.

RUTH MITCHELL and FIONA PLENDERLEITH (The James Hutton Institute, Aberdeen, UK) **Ash dieback – consequences for biodiversity and mitigation strategies**

Ash trees have 955 associate species with the following groups as obligate species on ash: 11 fungi, 30 invertebrates, and 4 lichens. A further 62 species are highly associated with ash trees. The consequences of ash tree and habitat loss were studied and modelled:

- For the obligate micromoth *Prays fraxinella* (ash bud moth) genetic diversity was high even in areas of fragmented woodlands.
- Mitigating the effects of the loss of ash trees, in terms of hosting the associated species may be partly achieved by other species such as oak, sycamore and elm. Tree species mixtures may be able to support 77% of ash-associated species.

VALENTIN QUELOZ (Swiss Federal Institute for Forest, Switzerland) **Assessment of different resistance testing assays for the ash dieback pathogen**

By 2008 ADB was in all parts of Switzerland. The initial response was to ban imports and now it is resistance monitoring. They compared different controlled methods of challenging ash trees with the pathogenic fungus to see which method best reflected field observed tolerance and susceptibility.

- 20 genotypes were selected from 10 forest sites; 10 showing field tolerance and 10 susceptibility and propagated by grafting.
- Stem inoculations were more reliable in showing disease reaction than inoculation of leaf rachises and showed good correlations with field observations even though the test takes 3 months.
- Rachis inoculation required a month to run but results were less consistent with field observations of each cohort of trees.
- More male trees appear to show resistance compared to females
- Some field selected susceptible trees showed as resistant to ADB in the inoculation tests and this may indicate that their field status was caused by agents other than ADB.

JAMES BROWN and Elizabeth S Orton (John Innes Centre, UK) **Heritability of resistance to ash dieback in the natural environment**

Severity of ADB in the landscape is quantitative ranging from severe to mild and strongly affected by local conditions such as site exposure, dryness, tree density and rainfall. Heritability of ADB tolerance is a function of genetics and environment. Environmental effects can be minimised by studying coppice shoots (all are replicates of the same genotype) from different trees in coppiced woodlands:

- There was little evidence of site differences in coppiced woods for disease incidence
- Heritability was estimated to be moderate to high i.e. 50-60% of the variability in health response could be attributed to genes rather than environment.
- When selecting for tolerance to ADB the selection intensity should be high because genetic correlation for molecular markers would be low for traits such as disease tolerance which is a polygenic trait.
- The breeding approach suggested was to select for moderate resistance (not extreme) among mother trees that are used for progeny testing followed by strong culling of

susceptible trees in progeny (90%). Thereafter the progeny from the remaining trees will express high disease tolerance

Relevant posters

JIE HUANG et al., Teagasc, National Botanic Gardens, Glasnevin and TCD, Ireland
Assessing the genetic diversity of selected alder plus trees in the existing Irish gene bank and seed orchards

Introduction

A clonal collection of 105 selected alder plus trees with Irish origin was established in Teagasc Oak Park centre in 2016. Exploring the genetic diversity and population admixture of selected plus trees for breeding programme is essential for maintaining the long-term sustainability and resilience of the alder improvement and forest reforestation program, as well as to guide better strategies of *in situ* and *ex situ* conservation. Questions posed: What is the genetic diversity of the selected alder plus tree collection in Teagasc gene-bank? How is the population structured within this collection? Are the selected trees genetically closely related to each other?

Results

The heterozygosity of 105 selected alder trees is lower than that found in other studies using SSR markers (Cubry *et al.*, 2015), this is likely due to use of different genetic markers used. Little inbreeding and population differentiation were observed in this collection and the collection is genetically admixed. Genetic parameters on economically and ecologically important growth traits (e.g. height and diameter breast height (DBH)) will be assessed to understand the heritability and genotypic & phenotypic coefficient of correlation using half-sibling progeny.

T. BYRNE et al., Teagasc, TCD, Ireland. Georg-August Univ Gottingen
Breeding Ash (*Fraxinus excelsior*) for tolerance to Ash dieback

Two Irish gene banks containing 208 and 165 genotypes were scored for susceptibility to ash dieback disease using a modified scoring Strategy. Disease assessment was conducted in Winter and Summer of 2022 and 2023. K-means clustering shows four optimal categories that genotypes can be classified based on scoring. It revealed that 25.75% of genotypes can be classified as tolerant trees. Trend analysis reveals a reduction in tolerant genotypes over time.

Results from the field trials so far have shown a wide range of responses to ash dieback disease, however approximately a quarter of the genotypes show higher level of tolerance. Bi-annual assessment of disease, along with growth phenology will allow for the expansion of the breeding program to produce tolerant and improved genotypes. Through the development of molecular and biochemical markers we can supplement the scoring system. Screening at the seeding stage for disease tolerance, allows for the rapid rotation of genotypes for the breeding programme.

E HALL, et al., *AFBI (N Ireland), FGRT, Teagasc, UEX.*

Genetic pathway to tolerant ash trees and their adaption for forestry, agroforestry and hedgerow systems in the island of Ireland.

Ash dieback disease is ravaging the native population of ash trees in forestry, agroforestry and hedgerow habitats in the island of Ireland. The pathogen is now past the point of eradication, being present in the whole island. The control and mitigation efforts against ash dieback have tended to focus on the pest/pathogen and frequently neglect the importance of the intra specific susceptibility of its host. Tolerance to ash dieback disease is found in natural populations with a broad genetic base and this tolerance is heritable and thus, breeding for tolerance can be feasible. Ash research in the island of Ireland will therefore assess the potential of the existing genetic variation for tolerance to disease, and additionally the selection of disease-free trees in highly infected plantations, as well as control measures, that could align genetic variation within tree species against the impact of external environmental variables. However, depending on their genetic variation, efforts might be made in subsequent breeding and testing improvements. In this context, we will identify, monitor, propagate ash genotypes by grafting and establish gene-banks and field trials of potentially tolerant ash using the cohort of the most durable tolerant trees, as parent trees generate reproductive materials in dedicated plantings called seed producing orchards. These seed orchards will produce seed, which will be used for generating healthy ash trees in the island of Ireland.

Combined, the research will bring multi-disciplinary teams together to provide a whole system approach for ensuring the survival and sustainability of ash in the Irish landscape and address its management to ensure the preservation and restoration of ash as a native and commercial forest tree species on the island of Ireland. This project is supported and funded by DAERA and DAFM.

Gerry Douglas

November 2023