Chalara in Europe

Reports on European Workshop Meeting on Chalara
Cost Action FP 1103, Fraxback 16–18 September 2014, Palanga, Lithuania

Gerry Douglas, Teagasc

This meeting was attended by 95 delegates from over 30 countries to discuss research in Chalara. See COST Fraxback website: www.**fraxback**.eu.
Some of the most relevant contributions with regard to Ireland are summarised below:

- **Andrew Cotterill, DEFRA (UK)** andrew.cotterill@DEFRA.gsi.gov.uk

Using analysis to help prioritise tree health policy and management decisions: a cost-benefit approach for Chalara.

The UK strategic plan is to reduce the rate of spread of Chalara in the UK by surveillance, tree removal in selected counties, resistance breeding and engagement with the public. They have reviewed the monetary value and environmental impact of losing ash in UK forests. The UK has 3 million hectares of forests and the commercial timber value of their 142,000 ha of ash forests is £20m pa. In addition the environmental value of the ash forest is estimated at £150m pa. This combined value of £170m pa has been determined from extensive studies and careful analysis and is widely accepted.

- **R. Enderle, FBW/FVA (Germany)** rasmus.enderle@forst.bwl.de

Genetic resistance against ash disease in autochthonous Fraxinus excelsior clones in Germany.

They examined 246 genotypes of ash in seed orchards which originated from different German populations, and scored the level of Chalara by measuring crown defoliation (%) as an indicator of overall tree vigour and the quantity of epicormic short production as a measure of susceptibility to Chalara over two years (2012 and 2013). There were large differences in the level of disease between different orchards and individual genotypes. Susceptibility and tolerance to Chalara is genetically determined. Crown defoliation ranged from 17% to 35% and the portion of epicormic shoots was from 20% to 48% between different orchards.

Every genotype was affected by Chalara, some to a high degree, others to a minor degree. There were large differences between genotypes in the degree of infection. The level of susceptibility of each genotype remained the same from year to year. Further investigations are to determine if the least damaged genotypes remain healthy in future with the view to retaining a sufficient number of genetically diverse and tolerant genotypes for breeding resistant trees.

- **M.M. Tollefsrud (Norway)** mari.mette.tollefsrud@skogoglandskap.no

Genetic structure in ash populations on the Northern Range margins.

Chalara has progressed in Norway from the East and is steadily moving northwards along the coasts in fragmented populations. The population in Norway was shown to be derived from refugial populations in South Eastern Europe after the last glaciation. Norwegian ash has a complex population structure accompanied by a decrease in genetic diversity the further north one goes. They found that the Northern population and populations which have increased genetic relatedness are more susceptible to Chalara disease.

- **T. Kirisits, IFFF (Austria) (Two presentations)** thomas.kirisits@boku.ac.at

1. *The Silvicultural importance and ash dieback susceptibility of Fraxinus species in Austria and recommendations for disease management.*

Common ash *(F. excelsior*) is the second most important broadleaf species in Austria. It is severely affected by disease. *F. angustifolia* is most susceptible. *F. pennsylvania* is occasionally affected and *F. ornus* (flowering ash), has shown no symptoms so far. In inoculation tests, one selected 'resistant' genotype (clone 8) was shown to be tolerant of Chalara. Management guidelines in Austria are:

- Cut down severely affected stands
- Leave and maintain any 'tolerant' trees which have: negligible crown dieback, no stem/collar lesions, dense foliage, especially late in the season and a good overall condition of the tree.

2. *Ascocarp formation of Hymenoscyphus fraxineus on several year-old rachises and on woody parts of* Fraxinus excelsior.

The spore producing bodies (ascocarps) are found on decaying leaf petioles (rachises). The spores germinate on the leaves causing leaf lesions on top and on the underside a brownish stain can be seen on the rachises and veins of the leaves. The fungus then develops in the petioles and forms a pseudosclerotia structure which is black in colour. These structures can remain dormant for several years, for example, if kept dry. The pseudosclerotia then develop into the spore producing ascocarp structures under normal environmental conditions in the year after the infected leaves are shed.

This study has shown that ascocarp formation occurred under semi-notural and field condition for a period of up to five years after the leaves had been shed. This finding suggests that old rachises with pseudosclerotial structures are an important reservoir of inoculum of the ash dieback pathogen in the leaf litter.

Formation of spore-producing ascocarps on woody twigs was also studied. Field observations suggest that sporulation on dead shoots, stems and at soil level (root / stem collars) of trees was generally rare. From incubation studies it was shown that 81% of woody stem pieces (which had pseudosclerotias) developed apothecia in 2011 and 10% in 2012. It was concluded that sporulation on woody stems is of relatively low importance (compared to leaf sporulation) in areas where the pathogen is well-established. However, stem infections are a likely source of infection for introducing the pathogen to new areas.

- **H. Tiefenbacher, Private Forest Estate (Austria)** tiefenbacher@grafenegg.at

Tree dieback: consequences for an Austrian private forest estate

Manager of a 5,000 ha. forest. An assessment of 6,000 ash trees showed that 75% of all trees were slightly or not affected by Chalara, but badly affected trees were found in all stands examined with crown defoliation worse in young stands. Ash mortality ranged from 1.5% to 5.5% and the impact of continued attrition will be devastation for the forest in a few years. Silvicultural options for managing this situation are very limited to mitigate economic risks. Feasible options on species with lowest risk include: walnut, sycamore, plane, robinia and ailanthus. However, many of these are not acceptable for Natura 2000 areas.

- **V. Cermakova, Beno University (Czech Republic)** Vendula.Cermakova@mendelu.cz

Occurrence of putative dsRNA mycoviruses in Ash Dieback Causal Agent

Viruses (mycoviruses) have the potential to reduce the virulence of fungi which attack trees such as the virulence of Cryphonectria fungus on Castanea trees. 106 isolates of Chalara were analysed from 8 country sources for the presence of RNA (ds RNA) molecules which are indicative of the presence of mycoviruses. Three different ds RNAs were confirmed in 32% of the analysed samples. The discovery of ds RNAs which reduce the infection potential of Chalara remain to be found.

- **R. Mitchel & A. Broome**, James Hutton Institute and Forest Research respectively, UK Ruth.Mitchell@hutton.ac.uk alice.broome@forestry.gsi.gov.uk;

Ash dieback in the UK: identifying ecological and conservation implications and potential management options.

Ash constitutes 11% of the total broadleaf area in the UK, on 142,000 ha. The ecological value of ash in terms of its function of nutrient cycling, litter quality, leaf decomposition rate and topsoil pH were assessed. For all of these parameters, ash was the superior species with the exception of leaf decomposition rate, where ash was equal to sycamore. The potential ecological impact of ash dieback on 955 species which are associated with ash was assessed. Common ash is a host for 12 birds, 28 mammals, 58 bryophytes, 68 fungi, 241 vertebrates and 548 lichens.

Among these, some species were 'obligate' associates on ash (i.e., ash was the only tree species on which they form a biological association). Forty-five 'obligate' species were identified, consisting of 11 fungi, 30 invertebrates and 4 lichens. The next level of dependence/association with ash was 'highly associated' species and in this case, 62 species were highly associated with ash. No single other species of tree (out of 48 species assessed) can act as host for all of the ash associated species. Oaks can host 69% of ash-associated species and mixtures of various tree species have potential to host an even higher percentage. However, alternative species which support the greatest

number of ash associated species such as oak or beech will not replace the ecological functioning capacities in ash woodlands. It was determined that ash trees interact with the environment in a unique way, especially in regard to nutrient cycling. For ecological functioning capacity, alder, rowan and lime are most similar to ash, sycamore is intermediate and oak and beech are least similar. With regard to ash - associated species which are at risk (of extinction), the greatest number of these species can be supported by a mixture of elm, oak, hazel, aspen and sycamore.

In a study of 15 sites, a total of 274 species were found associated with ash trees and of these 116 were vulnerable to the loss of ash in the woodland environment; including 17 lichens, 17 fungi and 14 bryophytes. For example, the moth Ennomus fuscantaria lives only on ash in woodlands. Most of the sites examined had alternative host species but at low abundance so management interventions on such sites are required to increase their abundance or supplement their species diversity. It is anticipated that management options can somewhat mitigate the loss of ash from woodlands, but 'obligate' ash associated species will eventually be lost from sites as the ash population dies out.

- **L. Havrdova, Silva Tarouca Inst. (Czech Republic)** havrdova@vukoz.cz

Environmental factors affecting the impact of ash dieback.

Ash dieback was identified in 94% of the 80 study plots within forests among different vegetation types. The average damage was 30%. The percentage of crown withering was 15.2% in riparian stands and 14% in alluvial stands of ash with alder. West oriented stands were most affected and trees on slopes were less affected. Trees in stands with diseased neighbours and high humidity were most affected.

- **L. Kelly, Queen Mary University, London (UK)** l.kelly@qmul.ac.uk

Fraxinus genomics

Genomic research is undertaken with the aim of finding gene sequences associated with susceptibility and with tolerance to Chalara. A reference genome sequence for a homogozygous ash tree is available. Re-sequencing individuals from each of 37 European provenances has been done at low coverage, and 90-92% of the records have been shown to map to the reference genome. Genome sequencing of 35 species of Fraxinus is underway to analyse for genes with a potential correlation with disease tolerance. In this latter case, oriental species appear tolerant to Chalara and may have co-evolved with the fungus.

The study aims to identify candidate genes associated with disease tolerance and patterns of molecular convergence of gene sets among the different species as a means of finding resistance genes.

- **G. Thorp, FERA (UK)** Gilli.Thorp@fera.gsi.gov.uk

Mitigation of impacts of ash dieback in the UK – an investigation of the epidemiology and pathogenicity of H. fraxineus and development of methods for detection and containment of disease spread.

Dieback is well-established in the wider environment on the East of the UK. It has a wide temperature range for infection (10 to 25°C) and the production of spore producing bodies (apothecia) occurred in the range of 10 to 30°C after 6 to 15 days of incubating leaf rachises. Spores are produced from July with peaks in production during August and September. They used spore traps to show that there were big differences between sites in terms of spore production. The peak of spore production was from 5.00 am to 8.00 am with 4.5 million spores detected per day. Peaks in spore production were associated with rainfall events. Treating the leaf litter with fungicides, experimental products and urea, resulted in some reduction in apothecia production with all treatments.

Field visit to ash dieback affected forests in the Klaipeda region, Lithuania

Ash dieback was first noticed in 1995-96 in Lithuania. The disease is now severe in all parts of the country. Stands of ash of all ages are being felled so as to recover some value from the trees, before the trees die and wood rots set in. The area of *F. excelsior* stands has decreased from 50,800 ha in 1995 (before the occurrence of dieback) to 36,300 ha in 2011. Data collected from permanent monitored plots show about 10% of ash trees die out every year and affected living trees grow poorly. The mean proportion of sound-looking trees in the monitored plots has dropped from 29% in 2008 to 7% in 2012, while the proportion of dead trees has increased from 0% to 45% in the same period.

Dieback disease has completely destroyed the one and only stand of ash which was designated as a seed stand and 5 out of 11 former ash genetic reserves have also been destroyed. In most of the remaining ash stands, the density of viable trees which flower and produce seeds is extremely low (3–5 trees per ha).
Younger stands (age <50 years) are more intensively damaged than the older stands (>50 years), however the overall stand health is deteriorating irrespective of stand age.

Observations made at the field stops:

Stop 1 was an uncut stand consisting of 20% ash of 130 years old (7.7ha). These large trees had dieback of shoots in the crowns and some trees were dead. No young saplings were observed.

Stop 2 was a 130 year old stand that had been clear cut in January 2013. In Lithuania ash forests are normally restocked after clear felling by the natural regeneration of seeds produced in previous years by the large trees. Natural regeneration after clear felling normally produces well over 100, 000 saplings per ha. It was estimated that after clear felling, in this site, there was approximately 200-300 saplings regenerating per hectare. This low number of regenerating saplings was due to reduced seed production by the Chalara affected trees in the years before felling and the presence of Chalara which killed young seedlings.

Stop 3 was a clear cut site of 0.8 ha area in a 4.6 ha stand area with 40 % ash in composition, 80years old. It was felled in 2012. We found very little natural regeneration from saplings or stump sprouts.

Terms:
Chalara fraxinea is the vegetative state of the fungus (anamorph stage)
Hymenoscyphus pseudoalbidus is the sexual stage of *Chalara* (teleomorph stage); it is now re-named as *Hymenoscyphus fraxineus.*
Ascocarp: (used interchangeably with 'apothecia') these are very small (2.0mm) white mushroom- like body that produce infective spores on fallen leaves, in the year after they are shed

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