Chalara in Europe

Reports on European Workshop Meeting on Chalara
Cost Action FP 1103, Fraxback 12-16th of April 2015, Hotel "Cavtat", Dubrovnik,
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This workshop exchanged research information on Chalara as well as on Dutch elm disease. It was attended by 100 delegates from over 30 countries. Some of the most relevant contributions with regard to Ireland on Chalara are summarised below:
See COST Fraxback; website: www.**fraxback.**eu.

NOTE:
'Chalara' is accepted as the commonly used name for the fungus which causes Ash Dieback Disease (ADB).
The scientific name for the vegetative state of the fungus (anamorph stage) is *Chalara fraxinea*.
The scientific name for the sexual stage of this fungus was originally given the name *Hymenoscyphus pseudoalbidus* (teleomorph stage)
That name has now been changed, so the official name for the sexual stage of the fungus is ***Hymenoscyphus fraxineus.*** In most scientific reporting the abbreviation *H. fraxineus* is used. The abbreviation 'ADB' is used for Ash Die Back

Summary of Presentations:

Can *Hymenoscyphus fraxineus* be eradicated from Ireland?

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A summary of the situation in Ireland was presented and it was pointed out that a truly successful eradication of any pest or pathogen is 'very rare' once it becomes established. However,the author was hopeful that since our prevailing winds are from the west/south west that "the incursion of airborne spores was unlikely to occur'' It was also reported that there have been no detections of pathogen spores in spore traps during 2014.

Magnitude and temporal dynamics of declining health of ash trees due to infection by *Hymenoscyphus fraxineus*

Kjaer E. et al., (Denmark)

The average health status of Danish ash has declined dramatically over the past decade with high mortality. The health and mortality of different stands aged between 10 and <100 years were monitored from 2010 to 2014. In 2010, symptom free trees varied among sites from 0 to 26% while the corresponding frequency of heavily infected trees varied from 37 to 70%. ADB arrived in 2002 and within a few years infection appeared throughout Denmark. In young trees (33 years old) the percentage of healthy trees went from 37% in 2010 to 20%

in 2014 and mortality was 26% in 2014. For 50 year old trees 78% were healthy in 2010 and this declined to 47% in 2014 with zero mortality. For 55 year old trees, health status went from 36% to 8% in this same four year period, while for old trees (100 years+), health status was 30% in 2010 and declined to 18% in 2014 with 10% mortality.

There was a rapid decline in health and high mortality in young trees and this decline continues. Some trees (approx. 25%) are relatively healthy. This research group have identified trees with apparent tolerance / resistance to Chalara and they have propagated them by grating and also collected seeds from 180 of these selected trees. High disease pressure is the best environment for selection of tolerant trees and young trees are the best for selection since they appear most sensitive. Leaving resistant trees in the stands leads to pressure on those trees since they may suffer stresses such as a higher water table once the affected trees are removed.

Tissue specific colonization profiles of the ash dieback pathogen *Hymenoscyphus fraxineus* in the stems of Norwegian and Ukrainian *Fraxinus excelsior* saplings (FP1103 FRAXBACK STSM)

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Only central Norway remains Chalara free. Disease is also present in Ukraine. Microscopic examination of disease progression showed it moved from the pith to the bark area in stems with abundant colonisation of xylem and parenchyma. The pathogen was also found in the pith of non-symptomatic stems, so the pith infection is an important source for radical spread of the disease.

Vegetative compatibility in *Hymenoscyphus fraxineus*

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The fungus shows vegetative incompatability between strains when grown together in culture. This mechanism has evolved to define individuals, promote sexual outcrossing and confers a restriction of virus spread among different vegetative lines. Chalara fungus has been shown to harbour fungal viruses, so these must be transmitted via sexual ascospores rather than by vegetative matings / coalescences.

LIFE+ ELMIAS project aimed at saving ash and elm in Gotland Island, Baltic Sea, Sweden

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Ash dieback has spread annually in Sweden. Their aim is to find tolerant genotypes in severely damaged ash stands and to use these for breeding. So far 135 trees have been identified and seeds have been collected to undertake further screening of material in Gotland, a Swedish island in the Baltic sea.

Tree health and biosecurity: what can social research tell us?

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A survey on the awareness of Chalara in the UK indicated that 74% of people expressed concern but 81% said they know little about Chalara. Among forestry professionals; only 20 – 30% said they were knowledgeable regarding this disease.

What makes the ash dieback fungus *Hymenoscyphus fraxineus* pathogenic?

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The genomes of *H. fraxineus* (pathogen) was compared to the non pathogen, and the close relative the *H. albidus*. They examined 14,000 genes and found 11,000 genes common to both species with 2423 unique to the pathogen and the functions of these are being studied.

Genetic analysis of European common ash (*Fraxinus excelsior* L.) populations affected by ash dieback.

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This lab has analysed the genetic diversity in host populations affected by ADB, among susceptible or less susceptible trees. They found a high genetic differentiation between populations with markers in chloroplast DNA separating North and South populations in Bavaria. They found that for the less affected trees by ADB, that they were more heterozygous i.e. more genetically diverse. There was not a significantly lower level of genetic diversity in the less susceptible trees, therefore it is safe to build a breeding programme using these trees.

Prevalence of collar rots in ash under different site conditions

Berthold Metzler and Rasmus Enderle

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This group reported that ADB is now prevalent in Germany and salvage fellings now accounts for 50% of all ash harvested, i.e. 1% of the standing volume. They noted an increase in collar rot (rotting of the trees at ground level leading to their falling over). They found 15% of trees with collar rot in provenance trial trees and these trees did not display symptoms of ADB in the crowns. Over 4 sites, 60% of the trees had collar rot on wet sites and 20% on the drier sites, and pole size trees were more affected than timber size trees. On stagnant wet sites (Carex indicator), 70 – 90% of pole size trees had collar rot. Infection takes place initially by *H. fraxineus* followed by *Armillaria gallica*.

Three close relatives of *Hymenoscyphus fraxineus* identified on *Fraxinus platypoda* and *Fraxinus chinensis*

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H. albidus is a non pathogenic relative of the pathogen *H fraxineus*. This research group has studied other relatives of *H. fraxineus* i.e., *H. linearis*, found on rotting petioles of *F. platypoda* in japan while *H. koreanus* and *H. occultus* sp nov were found on F. chinensis subsp. rhyncophylla in Korea. The asiatic species of ash such as *Fraxinus mandschurica* and *Fraxinus platypoda* are a host for Hymenoscyphus species but they are not pathogenic on them. *H. linearis* is not pathogenic on common ash *F. excelsior* but *H. fraxineus* is pathogenic on several ash species.

Whole Genome Sequencing of *Fraxinus* Species

Laura J Kelly et al., [1]*School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK*

She reported on the sequencing of the whole gerome of *Fraxinus excelsior* and a model has provided evidence for 43,296 genes and 59,000 transcripts. The analysis of 35 ash species is also underway and screening for susceptibility and tolerance to ash dieback. The ultimate objective is to identify gene sequences for resistance to ash dieback as well as the Emerald ash borer, which is a serious insect bark beetle in the USA.

Local Spatial Population Dynamics in the Ash Dieback Pathogen *Hymenoscyphus fraxineus*

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They examined the genetic diversity in the pathogen *H. fraxineus*, between different types of ash stands; i.e. a monoculture a two species mixture and a 4 species mixture, which originated 4 – 6 km apart. They found no genetic differentiation between the different sources of the fungus at the different sites.

Natural regeneration of common ash *Fraxinus excelsior* L. stands in Latvia

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They studies natural regeneration in ash stands affected by ADB. One third of all ash forests in Latvia are dead and ash is no longer the dominant tree in the forest canopy. Ash regeneration was significantly affected by the dominant tree type in the laver over 7.0 m in height. In ash dominant sites sapling density was 10,477 trees/ha. They found the greatest degree of damage due to ADB on wet sites and sites with high shrub cover. Almost 90% of saplings up to 20 cm high were healthy but for trees over 2.0m in height, the percentage healthy was 50%.

Endophytic fungi in *Fraxinus excelsior* shoots and their antagonistic activities against *Hymenoscyphus fraxineus*

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They studied endophytic fungi and bacteria i.e which reside naturally within ash trees and their effects on *H. fraxineus in vitro* from healthy and diseased ash. They obtained 921 colony isolates and 58 of these were fungi. There was no significant difference in the fungal community isolated from healthy and diseased trees regarding species number, but higher numbers of fungi could be isolated from winter shoots and it was speculated that the capacity of *H. fraxinus* to spread within winter shoots may be due to the absence of some fungal species. Two isolates of *Crocicreas* spp. and one of *Botrytis cinerea* had some antifungal activity against *H. fraxineus.*

Impact of ash dieback on individual competitiveness in dense natural regeneration

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Resistance to ADB is quantitative in nature, therefore, even for individuals with high resistance, we can expect they will be somewhat affected during their lifetime. Disease severity was recorded in naturally regenerating sites (up to 150,000 trees/ha). Tree height was taken as an indicator of the competitive capacity of these regenerating trees in 3 stands with 34 – 64,000 trees/ha. Among the 3 sites only 7% of trees were unaffected by *H. fraxineus*. From summer 2013 to winter 2014/15, the disease severity increased; smaller trees were affected and the number of ash in the stands decreased by 20.4%. Height growth in moderately affected trees was not reduced compared to completely healthy trees but there was a significant height reduction for trees which had over 50% of their twigs affected by ADB and such trees lost 30 – 40% of their height compared to less affected trees.

Virulence of *Hymenoscyphus fraxineus* isolates from Lithuanian (post-epidemic) and Swiss (epidemic) populations

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They compared the virulence of isolates of *H. fraxineus* between strains isolated at the early stages of the epidemic and at later stages. Inoculation tests were made using isolates from (1) active stem lesions (from living trees) and (2) from fallen petioles (saprophytic phase). High infection rates were found for both strain types but there was no evidence that the organism is more virulent during the expanding epidemic phase compared to the later stages when the disease is widespread. There was also no genetic differentiation between Swiss and Lithuanian populations of *H. fraxineus* or between isolates from stem lesions and from petioles.

Are ash leaf endophytes endangered?

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They surveyed the fungal communities which live invisibly (to naked eye) within and on ash trees as well as on Sycamore and flowering Ash *(F. ornus*). They found 127 different fungi including ash canker *Venturia fraxinii, Diaporte* spp, *Mycosphaerella* and *Colletotrichum*. Fourteen fungi were found in common on ash, flowering ash and sycamore but three species were host specific to ash and therefore are likely to be threatened with extinction if the entire ash population became extinct. It was found that *Venturia* from *F. excelsior* and *F. ornus* inhibited the germination of spores of *H. fraxineus* in laboratory conditions.

What do we know about ash dieback on *Fraxinus ornus*?

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Common ash (*Fraxinus excelsior*) and *F. angustifolia* (narrow leaved ash) are highly susceptible to *H. fraxineus* and are severely affected by ash die back. *F. ornus* (flowering ash) appears to be resistant to *H. fraxineus* as there have been no reports in nature of diseased trees. However, artificial inoculations produce lesions but they were smaller than on *F. excelsior*, so it was concluded it was weakly susceptible. Leaves and leaflets may show symptoms but the fungus does not appear to involve the stems.

Collar necrosis in ash stands affected by *Hymenoscyphus fraxineus* in Belgium

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It was noted that ash stands with ADB have a rotting at the junction of root and shoot, i.e. root collar. In examined stands, collar necrosis increased from 31% in 2013 to 55% in trees in 2014, and the shift was to larger trees. Out of 17 stands surveyed only two had no collar necrosis. The average crown defoliation increased from June 2013 to September 2014 and there was a significant correlation between the degree of defoliation and the proportion of trees with collar necrosis in September. Only 41% of trees with collar necrosis were infected by *Armillaria* spp. while 98% showed infection by *H. fraxineus*. Trees infected with *H. fraxineus* alone were found in the several different DBH size classes. Their conclusion was that *H. fraxineus* can cause collar necrosis even in large trees DBH >25cm.

The concentration of infective spores of *H. fraxineus* are 30 times higher at ground level (0.5m) compared to 3m. This is because the spores are released from fallen leaf petioles on the ground. Young trees have more stem lenticels and therefore a lower level of inoculum may be needed for infection to take place when compared to older trees with fewer lenticels; in this latter case a higher inoculum level may be needed for infection.

Screening and selection of common ash (*Fraxinus excelsior*) for resistance to *Chalara fraxinea* in Great Britain – The Living Ash Project

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Their objective is to find 400 tolerant ash trees for breeding. They have selected sites in the south and east England where *H. fraxineus* concentration is highest for screening material to identify resistant / tolerant trees. They have planted out 155,000 ash trees in screening trials (2 years old) on 14 sites in the UK in 48ha. This trial includes two provenances from Ireland. All 14 sites have been confirmed as chalara positive and they aim to identify tolerant trees over the next 2 years.

Associated fungal community of *Fraxinus mandshurica* in Far East Russia

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F. mandshurica is a species in Asia and the far east of Russia and the fungus *H. fraxineus* is a benign associate of this species but is an aggressive pathogen on our native *F. excelsior*. They examined the composition of the fungal community on *F. mandshurica* and found 49 taxa and several of them were in common with *F. excelsior* including *Phomopsis* spp., *Phoma* spp, *Cladosporium* spp. Some species such as *Coniozyma* spp were unique to *F. mandshurica* and may have a potential role in protecting this species. *Mycosphaerella spp* were the most dominant in species and they appear to be uniquely associated with *F. mandshurica*. The genetic diversity of the *H. fraxineus* population in Asia was significantly higher compared to the European population of this fungus. Asian species of ash such as *F. mandshurica* appear mainly unaffected by the many strains of *H. fraxineus* and this suggests that that genes for resistance may be found in Asian ash species such as *F. mandshurica.*

Root die back on ash trees

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ADB is present for about 7 years in the Danube region of Austria causing many young trees to die. In the past 3 years, crown rot has been observed and trees fall down spontaneously. In his estate he is cutting 6% of the stock p.a., whereas before ADB it was 1½%. The highly valuable large trees are being lost due to ADB and the loss to Austria is estimated at €230m irrespective of re-planting costs.

Development of a biocontrol agents for ash dieback using fungal endophytes

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This group is searching for bio-control agents for ADB. Over 80% of endophytes isolated from ash showed some biologically active against *H. fraxineus* in cultures among 250 endophytes which were isolated. Among those which showed strong inhibiting properties under lab conditions were *Venturia* spp. and *Phomopsis* spp. Among these fungal isolates only 5 of them were non virulent endophytes when grown with plantlets axenically cultured. In co-culture experiments four of these endophytes appeared to offer some protection against *H. fraxineus* and may have potential as biocontrol agents.

Development of an ash dieback (ADB) monitoring system for amenity trees in the city of Amsterdam

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The municipal population of over 18,000 ash trees in Amsterdam is being monitored for ADB. They have developed a workable system for assessing tree health and risk. They have assessed for defoliation level due to ADB and they have ranked the susceptibility of species from low to high accordingly: *F. pennsylvanica* < *F. angustifolia* < *F. america* < *F. ornus* < *F. excelsior*. Among clones of *F. excelsior* the ranking of susceptibility from low to high was: Geesink, Altena, Atlas < Westhof's Glory < Jasapidea, Diversifolia < Eureka < Pendula.

Health condition of ash and elm in Ukraine

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Ash covers 152,000 ha. 2.4% of the forest area and in 2010 5.6% of trees were affected by Chalara. They examined five provenances and found two without symptoms whereas the remainder had severe symptoms of dieback and mortality of some trees.

Situation with emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera, Buprestidae) in western Russia

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This group is studying the spread of the Emerald Ash Borer (EAB) which is a beetle which produces larvae under the bark of ash trees and kills them. EAB is found in 24 states of N. America and has caused $1.2 BN in damage. It is spreading outwards from Moscow since 2003 and kills trees outright. It has spread up to 500km in all directions from Moscow. All European and American species of ash are susceptible; only Asiatic species such as *F. mandshurica* and *F. chinensis* are unaffected and highly resistant. Their research is concentrated on finding parasitoids which can parasitise and kill the destructive larvae. Some promising candidates have been found.

***Hymenoscyphus fraxineus* in Russian Far East**

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Samples were collected in the Russian far east (Vladivostok and Khabarovsk) from leaves of *F. mandshurica* and *F. rhyncophylla* and the fungus was identified as *H. fraxineus*. The analysis showed they were similar to isolates from Japan. *H. fraxineus* was detected on shoots of *F. mandshurica* which had no symptoms of disease. These studies indicate that *H. fraxineus* is non-pathogenic but present on Asiatic ash species but it is a pathogen on *F. excelsior*.

Selection of alternative tree species: replication of ecosystem function or associated species?

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The suitability of alternative species to ash was assessed in terms of eco-system services, such as litter decomposition, top soil pH, nutrient re-cycling and the number of species which ash supports in woodlands. For these criteria, Acer species (& Alder next) were most similar to ash with beech and oak least similar. An assessment of the species mixtures indicated that 78% of the species supported by ash could be supplied by combination plantings of five other species, but could not fully substitute for ash as a host.

Marcais B. et al., (INRA France)

Basal collar rot has been found in 33% of all ash trees affected by ADB. When more than three quarters of the collar area is affected the tree mortality is 40-50%. In a survey of 42 stands, collar rots were found in 40% of trees affected by ADB. Overall, 63% of trees were affected by *H. fraxineus* in all stands examined. Armillaria was isolated from ⅔ of the stands examined but had no impact on the severity of collar necrosis. Several characteristics of stands were examined, dbh, pH, etc., but only topography affected disease severity. There was little collar necrosis on sloping sites. This may be due to a lower inoculum density with fewer leaf rachices accumulating. In conclusion, collar necrosis is a later stage of pathogenesis due to *H. fraxineus* in forests. Collar necrosis was also found in trees with healthy crowns and at least 55% of the trees with collar necrosis had healthy crowns.

Schoebel C. et al., (Switzerland)

Mycoviruses are found in all major groups of plant pathogenic fungi including *H. fraxineus* and may have potential to mitigate (reduce virulence) the pathogen effects. No DNA viruses were found but single stand RNA viruses were present in 91.6% of the fungal isolates from Lithuania, Switzerland and Japan. The same virus was found in fungal isolates from the North Alps and the South Alps indicating a natural spread of the pathogen in Switzerland rather than an introduction of a novel strain from elsewhere.

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