Chalara in Europe

Characteristics of *Chalara* disease, its status in Europe: summary report from a meeting of European experts

Based on abstracts and presentations of reports from various countries meeting in COST ACTION FP1103 'FRAXBACK', November 13-14th, Vilnius, Lithuania.

Gerry Douglas 29 Nov 2012

See COST action Fraxback website : www.**fraxback**.eu/

Main points:

1. Ash dieback disease is widespread in Europe; it has been reported on ash trees of all ages.

2. Mortality is greatest in saplings and young trees.

3. The fungus is not systemic within the tree. Trees are re-infected each year.

4. Disease spreads rapidly (35km / year). In Belgium in 2010 it was first observed in 6% of plots examined, by 2011 it was present in 66% of the plots. In observations sites in Germany the proportion of infected trees increased from 13% in 2007 to 88% in 2011. Mortality was 5 to 6% and radial increment was reduced by up to 26% and a corresponding reduction in basal area by 45%.

5. Disease is spread by ascospores which are produced on the petioles and on the rachises (decaying mid veins) of fallen leaves from June onwards.

6. In highly infected areas where there is a lot of spore release, the trees can become infected at the junction of stem and root and cause a collar rot at the base of the tree.

7. Trees have been found in highly infected areas which display resistance and the character of resistance has a strong genetic component so resistance breeding is feasible.

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

The host ash tree reaction to Chalara

- All ash species in Europe are susceptible, also exotic species of ash which are common in Europe e.g. *F. pennsylvanica, F. americana.* Some reports

suggest that *F. ornus* may be more tolerant and that *F. mandshurica* (chinese spp) is a host that is not badly affected by the diseases.

- Young trees are more likely to be killed than mid-aged and old mature trees.

- The wilting and death of new leaves has the effect of partially defoliating crowns each year. Growing branches die back to a main stem. Buds further back may sprout but the replacement shoots are not sufficient to provide enough leaf area to sustain the trees in the long run. Young trees die and older trees fail to make any significant increase in volume or diameter growth.

- Re-infection of new leaves occurs each year from spores produced from fallen leaves of the previous autumn.

- Trees can also be infected at the junction of the stem and roots. This results in necrotic areas under the bark and leads to the death of trees. In this case, infection is by the germination of spores at ground level and entry of the spores into the trunk through lenticels in the stem/root areas

- The fungus is not systemic within the tree, rather it re-infects the new leaves each year and causes dieback of the shoots.

- Chalara has been detected in / on ash seeds using DNA – PCR tests which are specific to the genes of the fungus. However, it is not clear if the test had picked up the presence of spores on the seeds or the presence of mycelium within the seeds. However, if young seedlings become infected they can be readily killed in the first year and seeds are a potential source of infection.

- During 2004 and 2005 phytosanitary checks in Belgium (Wallonia region) showed a good health status in ash woods. Surveys showed infection in 6% in monitored plots in 2010, on young naturally regenerated trees; by 2011 it was found in 66% of these monitored plots. Also in Belgium, collar necrosis was observed in 4 trees and 2 trees died in the course of one year in one badly affected observation plot of 250 trees.

- Reports from Germany indicated that tree mortality was rare especially in older age classes. However, in Lithuania where the disease is very widespread, overall, about 10% of trees die out each year. Studies in monitored sites from 2009, in Norway showed that a few infected trees could be found at the leading edge of the spreading fungus each year. Also, the health condition was variable within affected sites, ranging from very little infection to dead or dying trees. However, nearly one third of small trees were killed while for larger trees the infection level was unchanged from 2009 to 2012.

The Fungus

- Ash dieback is caused by a fungus. The vegetative stage of the fungus was identified as *Chalara fraxinea*, the sexual stage was identified as *Hymenoscyphus pseudoalbidus*

- Mycelium of *Chalara fraxinea* can be isolated from leading edge of the fungus in trees at the boundary of healthy wood with dead (necrotic) tissue. It can be cultured in the lab and it may produce conidiospores in culture. However, these spores have been shown to be non-infective.

- The fungal spores germinate and penetrates ash leaves. It then produces a black sclerotium on petioles and the leaf rachis. Ash leaves are compound in organisation. They have a petiole and 5 to 7 leaflets. The leaflets are connected together to the main vein of the leaf called the rachis. When ash leaves fall and decompose the leaflets are digested first, the main vein (rachis) is more resistant and Chalara develops within and on the rachises where it forms sclerotia.

- The fungus can remain dormant in the form of these sclerotia for at least two years and can be activated by moist conditions.

- In the summer after leaf fall the sclerotia on the fallen infected leaves produce tiny white mushroom-like bodies (ascocarps) which are just 2-3mm wide. These ascocarps produce sexual ascospores over a long period from June to October) with a peak in spore production each morning..

- Spores are forcibly released into the atmosphere and are highly infective on leaves causing dieback. *See video of spore release on fraxback web site* www.**fraxback**.eu/ *(videos).*

- Each ash leaf can have 20 mature ascocarps per petiole and each can release on average, 1476 ascospores per hour.

- All details of the life cycle is not confirmed. It is understood that the sclerotia may produce specialised conidospores which may become the male spermatia. Also, the oogonium (tricogyne) is produced in the sclerotial matrix and conjugation of spermatia with female oogonia occurs i.e. sexual fertilisation. This leads to production of infective ascospores in the ascocarps.

- Where the virulent *H. pseudoalbidus* occurs it appears to have replaced the harmless fungus *H. albidus* which can be found in decaying leaves of ash but this latter fungus does not cause disease. These two fungi are closely related and can be distinguished by morphological features.

- The origin of virulent *H. pseudoalbidus* is unclear. It may be a mutant of the harmless *H. albidus* or it is perhaps a new species of fungus that came into Europe from the East.

Reports on incidence and research on *Chalara* in several countries

Austria
First symptoms noted 2005 with massive leaf shedding in late august -september. Intensive dieback of shoots and twigs noted in 2006. Symptoms observed in all Austrian provinces in 2008. Disease is more severe on wet sites, mortality common in trees up to 30 yrs old and occasional in old ash trees.

Belgium
First reports of disease in 2010 on young naturally regenerating trees and is now considered widespread throughput Belgium. They developed a method of spore trapping which showed greatest spore release in early july. They developed a DNA based real time PCR to confirm disease presence.

Bulgaria
Significant leaf withering first noted 1990 in monitored stands but has been attributed to various fungi (not Chalara).

Czech Republic
First symptoms mid 1990s with massive symptoms in 2003 and confirmation of Chalara in 2007 . Ash stands with alder in lowlands river areas are most badly affected.

Croatia
Tests results have revealed the presence of Chalara in the north and western parts of Croatia and the production of ascospores in leaf litter in the summer following leaf fall. So far seed orchards of *F. angustifolia* in the Sava river areas are unaffected.

Denmark
First symptoms noted 2002-2003. Entire ash stock is now affected. Initially damage appeared to affect young trees, now, older trees are badly affected. Their extensive research has shown:

- Large and significant variation in susceptibility among tested clonal material
- There was no effect of site location on susceptibility of clones indicating negligible environmental effects and strong genetic effects on resistance levels.
- Healthy trees (without dieback) had yellowing leaves earlier in the autumn than affected trees, suggesting that trees which shed leaves earlier (become dormant earlier) may be less vulnerable.
- Material that is less susceptible to the disease had a slower development of necrotic tissues after artificial inoculation of resistant plants. This indicated the value of artificial inoculations to identify material with increased tolerance and resistance.
- It appears that healthier clones are able to limit the growth and spread of the fungus and thereby minimize the occurrence of symptoms.
- Only 1-3% of seedlings from native trees were found to be healthy in tests for resistance. Offspring from 101 trees in progeny trials were assayed and

revealed the presence of substantial additive genetic variation with a high level of heritability. That means that resistance is likely to be passed on to a large extent to the offspring from resistant parents.

Finland

From early 2000s symptoms were observed in trees in the island of Aland in the Baltic sea and on the mainland in 2007. Today Chalara is to be found everywhere in Finland. Their research below is extensive.

- Incubating petioles at 20^0C will produce diagnostic spore-producing bodies (ascocarps/ apothecia) within 2 months.

- Ascocarps (spore-producing bodies) can develop in the first year after leaf shedding.

- If leaves are in dry locations Ascocarps (apotecia) can be formed in the second or in subsequent years.

- Petioles which bear apothecia can continue to produce spores in at least two successive years, and need moist conditions to produce and release the spores.

- The fungus develops best in tissues at lower temperatures and in co-culture with other fungi it can out compete other common fungi in competition experiments in which *Chalara* is co-cultivated.

- *Chalara* mycelium was shown to continue to grow and develop in leaf petioles and rachices at 1°C.

- Production of infective spore producing bodies in the leaf petioles occurred by incubating infected leaf petioles at 22°C for just 2 months. This is a method to determine if the disease is present in suspect leaves.

- Affected trees in four sites were photographed over four years and this showed disease condition varied from year to year.

France

First symptoms seen in eastern France in 2008, now spreading north and west.
Research has shown:

- Approximately 33% of affected trees have collar necrosis.
- They found no association of disease occurrence with soil type, stand origin (plantation Vs natural regeneration), pure or mixed stands.
- For the average affected tree about one third of the crown was dead.
- Spore trapping has shown spores everywhere in affected areas.
- Among the trees in provenance and progeny trials about 8% of trees appear resistant but will they remain so ?; monitoring is continuing

Germany

First symptoms seen 2002 then *Chalara* was recorded in 2007 in north eastern Germany and then in southern Germany from 2006. Fellings began in 2006 to recover salvageable wood. Monitored sites showed:

- In Bavaria in six pole stage stands, 26% of trees were dead while in mature forests 2% are dying.
- The most sever mortality was recorded in 10 natural regeneration areas where tree mortality was 43%.
- Data from a provenance trial with eight provenances over four sites showed the proportion of affected trees was 13% in 2007 and 88% in 2011. In the provenance with the lowest incidence of the disease, 69% of the trees showed symptoms while 95% of trees in the provenance most affected showed symptoms.
- Radial increment was diminished by up to 26% in severely damaged ash.

Greece—no records of Chalara so far

Hungary

- First identification of Chalara 2008. Subsequently found all over Hungary in 2012 including mountain areas. In one nursery all trees were affected.

Ireland

Monitoring for symptoms since 2008. *Chalara* confirmed in trees in an imported consignment of 30,000 trees in 2012.

- All trees of the affected consignment traced and destroyed
- Policy—the disease is transient, actionable, under eradication
- Systems for large scale vegetative propagation described as a tool to accelerate breeding and deployment of resistant material

Italy

2009 Chalara detected near Slovenian border.

- Present in *F. excelsior* and *F. angustifolia*
- It is moving westwards, 50 – 60 km/year
- Chalara now present north of river Po.

Latvia

- Disease very intense with young ash stands most affected.
- Intensive stand degradation noted at a rate of 651ha per year

Lithuania

- Dieback first noted 1995-96 now severe.
- Due to continuing sanitary fellings, 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.
- Disease has completely destroyed the one and only ash seed stand and 5 out of 11 former ash genetic reserves. Today, the density of remaining viable and flowering trees is extremely low (3–5 trees per ha) in most of the remaining (yet, severely damaged) ash stands.

- Younger stands (age <50 yrs) are more intensively damaged than the older stands (>50 yrs), however the overall stand health is deteriorating irrespective of stand age.
- 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 respectively increased from 0% to 45%.
- Breeding for resistance is well underway using material in three progeny trials in three different regions of Lithuania . In these trials, a total of 140 open-pollinated families from ten Lithuanian populations and 180 open-pollinated families from fourteen European provenances / populations are evaluated (Belgian, Czech, Danish, French, German, Irish and Polish).
- In 2012, a clonal archive and 6 clonal -seed orchards have been established aiming to test the performance (growth parameters and resistance to *H. pseudoalbidus*) of material derived from identified progeny-tested material with greatest resistance i.e. 28 Lithuanian and 22 foreign *F. excelsior* clones and 180 clones selected for resistance in natural populations.
- Further testing of the most resistant clones is being carried out through artificial inoculation with *H. pseudoalbidus*; the new cycle of selection shall provide a possibility of obtaining a set of genetically most resistant clones for crossings in tree breeding and for mass propagation.

Netherlands

No serious reports of dieback until 2010 and confirmation of *Chalara* in 2011. The most severe infestations are in the north eastern part of the Netherlands.

- Roadside trees seem not to be badly affected.
- In clonal seed orchards and trial plot disease range from 100% to zero among clones indicating tolerance may be determined by the genotype of the individual tree.

Norway

- Ash is a minor species, 0.2% of forest area
- 2007 first damage reports in forests
- 2008 first nursery damage reported
- 2010 spread rate for Chalara disease recorded at 30 km from source.
- Large monitored trees had a stable rate for defoliation from 2010 to 2012
- approx $^1/_3$ of smaller trees died
- very little of the benign fungus *H. albidus* found.

Poland

Symptoms known since 1992 *Chalara* first described in Poland in 2006

- Maximum damage level in 2002 with 70 – 80% of trees affected in Warsaw region, no mortalities
- Two genetic variants reported of *Chalara;* one associated with lowlands, one with uplands (not reported in other countries).

Romania

Symptoms of ash dieback have been observed and recently mass dying of ash trees was recorded in plain areas. However, presence of *Chalara* has not been confirmed

Russia

630,000 ha of ash. 0.09% of forest area.

- *Chalara* confirmed only in the region of Kaliningrad on the Baltic sea.
- Emerald ash borer beetle (*Agrilus planipennis*) has killed 20 million ash trees in the USA and Canada in less than 10 years.
- This beetle has been recorded around Moscow in 2004/2005 and is actively spreading at the rate of 10km/year around the city areas.

Serbia

- 29% forest cover (2.2 m ha) Ash is 1.8% of area
- Dieback observed, not confirmed due to *Chalara*

Slovakia

2004- first dieback noted in trees up to 30 years old

- 30,000 ha of ash in Slovakia, 1.5% of forest area
- Disease has moved from East to West
- R&D on management felling, and fungicides for nurseries

Slovenia

First symptoms noted 2006, progressive spread 2007 to 2009 with most severe damage in wet sites and mortality highest in saplings and young trees.

- On monitored sites, crown reduction due to *Chalara* was 7% to 39% and tree mortality less than 2%.
- Eight fungicides were tested in vitro and carbendazim and procloraz were most effective in suppressing mycelium growth as well as the suppression of the formation of spore-forming apothecia.
- Optimal growth of *Chalara* in vitro was at 20–22°C, no growth at 28°C or higher
- Experiments on apothecia development of *H. pseudoalbidus* on leaf petioles (rachises) showed the need for sunlight and moisture. Apothecia (spores-producing bodies) matured after two months at 22°C in a moist chambers.
- They recorded on average 20 mature apothecia per rachis. Average sporulation was 1476 ascospores per hour per apothecium.

Spain

Some trees with dieback symptoms have been observed close to Madrid but *Chalara* has not been identified nor have spores bodies of *H. pseudoalbidus* been produced in incubated petioles—monitoring continues.

Sweden

First symptoms noted 2001, and noted in most areas by 2005. By 2009, 25% of ash trees had lost 50% of their crowns.

- Sweden has lost 30% of its entire ash population in 10 years and the species is now on the red list of vulnerable species in that country.
- There is a strong genetic component in ash populations conferring resistance so breeding for resistance is feasible
- The fungus produces viridiol in the host tree which is toxic to ash tissues.
- *Chalara* was detected in ash seeds indicating they are a potential pathway for disease.

Switzerland
First record of *Chalara* in 2008, and within four years, found in most parts of Switzerland, the most badly affected area is around Zurich.

- Their research group studies the reproductive biology of *H. pseudoalbidus.* Their study on longevity and drought tolerance of *H. pseudoalbidus* in petioles showed that sclerotia can withstand drying for three months and sporulate even after two years in nature.

Turkey
Authorities there monitor forests and obtained1268 isolates from symptomatic trees, several fungal organisms were identified, including *Phoma, Phomopsis, Pestalotiopsis* and *Alternaria*, but not *Chalara*.

Ukraine
First symptoms noted 2010-11 in eastern Ukraine, *H. pseudoalbidus* was confirmed in six sites in three regions of eastern Ukraine.

- In nine monitored sites, crown decline of 20-80% were found in 2010-2012
- In western Ukraine ash mortality has been attributed to other causes, cankers, flooding and insects and has resulted in sanitary fellings.

United Kingdom
Ash occupies 130,000 ha, 12 million trees (5.5% of total forest area), mostly in the south and Wales; the north and Scotland has just 5000 ha. Majority of trees are in the 40-80 age class.

- First confirmation of *Chalara* in an import consignment from the Netherlands in February 2012 followed by detection in the wild in October 2012, in east Anglia.
- Intensive surveys underway on 10 km grids, including 4 woods / grid
- Policy is that eradication is not an option in England Wales and Scotland due to its widespread presence but limiting the spread is underway in each country by destroying trees in affected nurseries and plantations.
- Policy aim is to identify all potentially resistant trees and begin breeding for resistance.

Dr. Gerry Douglas (Irish representative on COST Action FP1103 'FRAXBACK')
Teagasc
Agriculture & Food Development Authority
Kinsealy Research Centre
Malahide Rd.
Dublin 17
Ireland
Phone: Direct line + 353 1 8459006
Phone: Switchboard + 353 1 8459000
Fax: + 353 1 8460524
e-mail: Gerry.Douglas@Teagasc.ie
Web : www.teagasc.ie