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Ash dieback on the island of Ireland

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Abstract
On the island of Ireland it is estimated that there are over half a million kilometres of hedgerows (400,000+ km in the Republic of Ireland (Rep. Ireland) and 113,000+ in Northern Ireland (Northern Ireland). Ash (*Fraxinus excelsior*) is the second most important component, after hawthorn (*Crataegus monogyna*), in large proportions of this hedgerow network. In the Rep. Ireland over 20,000 ha of ash have been planted since 1990, primarily for sawlogs and to provide material for the manufacture of hurleys, which are used in an important national sport called hurling, and for camogie sticks used to play camogie. Ash dieback, caused by *Hymenoscyphus fraxineus*, was first identified on the island in October 2012 and since then has been detected at 306 sites (195 in Rep. Ireland and 111 in Northern Ireland). In the vast majority of cases the outbreaks have been on young, imported trees planted within the previous 5 – 6 years and it was evident that the pathogen had been introduced on trees for planting. On a small number of occasions there was evidence of the pathogen cycling within a plantation or moving from the plantation to infect neighbouring hedgerow trees. One possible mechanism by which the pathogen can build up sufficient inoculum is by the formation of apothecia on infected woody tissue high up on the plants. Rep. Ireland and Northern Ireland have strict policies of eradication and containment, as set out in the All-Ireland Chalara Control Strategy. To date over 2.1 million trees have been destroyed as part of an eradication strategy. It is considered that this prompt and far-reaching action has had a significant impact, significantly mitigating and preventing the rapid establishment of the pathogen and limiting its spread. The interventions since the disease was first confirmed have helped to protect the considerable investment in ash plantations of the last 20 years. The pathogen has not, however, been eradicated from the island of Ireland and it remains to be seen how widespread, and how quickly ash dieback will become established on the island of Ireland. The latest figures from the Republic of Ireland are that 733 hectares of ash plantation has been reconstituted with another species as a result of Chalara and this has cost our state €2.6 million so far; in addition, Chalara has been found and confirmed in all 26 counties of the Republic of Ireland to a greater or lesser extent. As a result the current policies and procedures regarding Chalara are under review.

Introduction

Ireland is an island 21 – 170 km distant from the west coast of another island, Great Britain, which in turn is separate from continental Europe. The prevailing winds tend to be south-westerly i.e. from the Atlantic Gulf Stream. These two factors mean that the probability of the natural spread of non-indigenous pests or pathogens into Ireland is significantly less than in most other parts of Europe. However, once introduced, the mild, moist weather conditions favour the growth and spread of many fungal pathogens. The relative isolation means that Ireland has had a high plant health status: it is currently free of many of the pests and pathogens which are currently causing significant damage to crops and trees elsewhere in Europe, but is still vulnerable. This is reflected in the 'Protected Zone' status for many organisms harmful to plant health that the Republic of Ireland and the United Kingdom (Northern Ireland) have under the EU Plant Health regime. Furthermore, experiences from continental Europe may not be directly applicable to Ireland. For example, many pathogens and pests found in Ireland have been introduced on plants or plant parts in trade rather than having spread from neighbouring infected regions. The maritime climate of cool summers and mild winters, high rainfall and little snow contrasts with much of northern continental Europe where extremes of temperature and rainfall are the norm. Hence any actions against pathogen or pest incursions to the island need to be considered in the Irish context.

While the island is divided into two EU Member State administrations: the Republic of Ireland (Rep. Ireland) and Northern Ireland (Northern Ireland) (a devolved administration of the United Kingdom of Great Britain and

Northern Ireland), the benefits of an harmonised all-island approach with regards to phytosanitary matters are recognised at both policy and delivery levels. There is, therefore, developing, ongoing close cooperation between the plant health services and scientists in both jurisdictions, i.e. the Department of Agriculture, Food and the Marine, DAFM (Rep. Ireland) and the Department of Agriculture and Rural Development, DARD (Northern Ireland).

In recent decades there has been a significant increase in the global trade of plants and plant products. It has been recognised that this movement of plants poses a high risk of introduction of unwanted organisms into areas which were previously free from them. It has been suggested that woodlands and native plant communities in the UK and Ireland are currently suffering from plant pathogens introduced, often on living plants, by human activity (Brasier 2008). This author suggested that the potential for future damage may be large and is critical of the international regulations aimed at mitigating the risks of introducing diseases, which he considers to be highly inadequate. High volumes of many categories of plants for planting in Rep. Ireland and Northern Ireland are grown outside Ireland, usually within the European Union (EU), presumably for reasons of supply availability and price competitiveness within the European Single Market (ESM). In contrast, there are limited imports of plants or plant parts from third countries outside the EU, and the phytosanitary regulations governing these tend to be very detailed and aimed at protecting the integrity of the ESM. As will be discussed presently it is highly probable that ash dieback, caused by *Hymenoscyphus fraxineus*, was introduced to the island of Ireland in ash trees for planting, brought from continental Europe which, in turn, was probably subject to intercontinental infection, initially from the far East (Drenkhan et al. 2014).

European or common ash (*Fraxinus excelsior* L.) is the only species of ash native to Ireland and is one of the commonest trees throughout the island, possibly because it has the qualities of a pioneering species that is well adapted to niche opportunities in a largely pastoral landscape, with very few old forests. It was one of the comparatively few tree species to colonise Ireland post glaciation and before Ireland became isolated from the European landmass. Ash has important heritage and ecological values in the landscape, both as a hedgerow and woodland species. In the Rep. Ireland ash is a significant component in 91% of native woodlands.

Rep. Ireland has an active afforestation programme and prior to the first finding of ash dieback, ash represented 10% of this afforestation, currently around 20,000 ha. In the period 1990 to 2009, an average of 670 ha of ash plantations were established per year. This represents a considerable investment and a growing resource, the value of which is increasing significantly as it moves towards maturity. The most active period of planting was in 2003 and 2004 when 1,800,000 plants were required each year from nurseries, a level of demand that necessitated imports of plants. Average plant demand was 1,300,000 yr^{-1} in the period 1997 to 2007. The overall areas of ash in various age classes are shown in Table 1.

Table 1 Areas (ha) of established ash in plantations and high forest in the Republic of Ireland.

Plantation type	Private plantation ash (grant aided)	Private forest (not grant aided)	Public forest (Coillte)	Total area
Period covered	*1980-2014*	*1980-2013*	*1800-2013*	
Area (ha)	15,995	8,318	4,015	28,328

Sources: Coillte – Coillte Sub-Compartment Database; Private Grant Aided – Department of Agriculture Afforestation Data; Private Non-Grant Aided – Ireland's National Forest Inventory 2012. Private Forest (not grant-aided) is comprised of both afforestation, reforestation and natural regeneration, i.e. predominately includes old estates and farms

Between 1990 and 2012 approximately 13,000 ha of ash were planted under Department of Agriculture, Food and the Marine (DAFM) grant support schemes. Ash is grown commercially for its dense, strong, but elastic timber, with targeted end use of timber for flooring and furniture and as sports goods (hurls) and wood fuel (firewood, smoking wood and barbeque charcoal). Traditional sports use ash in hurling and camogie, which trace their origins back to the Bronze Age and remain an important part of Gaelic heritage. In the sport of hurling, players use an ash stick (hurl or hurley) crafted from the butt of the tree where the grain runs into the root. There is an annual requirement for 360,000 hurleys, crafted from 2,000 m^3 of ash wood valued in the region of €450 per m^3 for logs at roadside. Currently 76% of this wood is imported. It had been hoped that by 2020, Ireland would be self sufficient in ash for hurleys when maturing plantations reach the required dimensions of 18 - 28 cm diameter at breast height (dbh) i.e. 1.3 m and this was seen as an important market for plantation thinnings. The fear is that if the disease becomes widespread this market will be lost and plantation logs will only fetch firewood prices of €60 per m^3. In response to the ash dieback outbreak, a Statutory Instrument required hurley makers to import only planks and debarked logs which significantly added to their production costs. This order has been rescinded recently as the risk of movement of the pathogen on wood and logs is deemed to be very small.

Ash is a vital part of the Irish landscape. There are numerous large mature ash trees all over the island. These may be remnants of former hedges or they may have been planted as parkland trees. However, the real landscape importance of ash is in Irish hedgerows. Hedges form the majority of field boundaries and act as stock-barriers. It may be that hedgerows could be a significant conduit for the spread of the pathogen. It had been estimated that there are in excess of 300,000 km of hedgerows in Ireland (Anon 2015a), which cover approximately 1.5 % of the land area. However in a further intensive study, The Heritage Council of Ireland (2015) estimated that hedgerows in Rep. Ireland have a linear dimension of 400,000 km. From GIS (Geographic Information System) and Lidar (Light Detection And Ranging) studies, the total area of hedgerows, individual trees, small woodland patches and scrub covers 350,000 to 450,000 ha in Rep. Ireland (Black et al. 2014; Green 2010; Sansford 2013). In many parts of the country ash forms a major component of those hedges. County surveys of hedgerows have shown ash is the second most frequent species after hawthorn (*Crataegus monogyna* Jacq.). The results of these surveys can be found at the website of The Heritage Council of Ireland (Anon. 2015c). The frequency of hedgerows which contain ash was lowest, at 10%, in Co. Offaly and highest, at 68%, in Co. Leitrim. Ash can also develop into trees and of the hedgerows surveyed, those which have ash as trees, ranged from 28% in Donegal to 75% in Longford.

In Northern Ireland there are fewer woodlands with an ash component but ash is still very important as a hedgerow tree, both in coppiced hedges and growing as single stem mature trees. It is estimated that there are 113,648 km of hedges in N. Ireland (McCann 2012) of which up to 90% have ash as a component. In some situations the ash is coppiced as part of the ongoing management of the hedges, while in other situations the ash trees are allowed to grow into mature single-stem, large trees. As in the Rep. Ireland, there is an active afforestation programme delivered by the Forest Service of the Department of Agriculture and Rural Development (DARD) and part financed by the EU. In Northern Ireland, however, ash is rarely the dominant species and is often included to add diversity to planting schemes.

Ash has great biodiversity significance. In a recent study of the importance of ash to UK biodiversity, Mitchell et al. (2014) identified a total of 1,058 species associated with ash trees. These included birds, mammals, bryophytes, fungi, vascular plants, invertebrates and lichens. Of these 44 were obligate, ash-associated species including fungi, invertebrates and lichens with a further 62 species including fungi, lichens, bryophytes and invertebrates highly associated with ash. Lawrence and Cheffings (2014) conclude that the contribution to UK biodiversity by ash trees is important and this is demonstrated by the dependence of 106 species on ash trees and the habitat that these trees create. Furthermore, of the 536 lichens that grow on ash in the UK, 220 are nationally rare or scarce, and 84 have been categorised as under threat (Mitchell et al. 2014). It can be assumed that the biodiversity associated with ash growing in Ireland will be very similar.

As a component of hedgerows and non-woodland patches, ash is important for carbon storage. In a study of Rep. Ireland hedges these two categories have been estimated to have a total carbon sequestration potential of 4.98 t CO_2 ha^{-1} yr^{-1} (Black et al. 2014). It is clear that the potential loss of a significant proportion of the ash population in Ireland to ash dieback would have large environmental, biodiversity, landscape and commercial impacts.

Although ash enjoys a widespread distribution in Ireland, most sites are sub-optimal for growth as a dominant forest tree and the dominance of ash in the Irish landscape is probably a comparatively recent phenomenon associated with field enclosures at the beginning of the 19^{th} Century, as described for County Leitrim (Foulkes, 2006). In Europe its widespread success is also thought to be recent, reflecting its role in primary and secondary woodland succession, and is closely associated with forests dominated by European beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* (Matt.) Liebl.), pedunculate oak (*Quercus robur* L.), sycamore (*Acer pseudoplatanus* L.), black alder (*Alnus glutinosa* (L.) Gaertn.) or grey alder (*Alnus incana* (L.) Moench). Ash seems to become dominant where site conditions are less favourable for the associated species or where human manipulation of the environment occurs (Dobrowolska et al. 2011). Thus, the dominance of ash in Ireland may be due to lack of competition from other forest trees coupled with agricultural practices. Over time, if ash is lost from the hedgerows it would probably be replaced by other hedging species such as hazel. It is not clear if tree species such as sycamore would naturally become more common.

Ash dieback (*Hymenoscyphus fraxineus*)

Ash dieback is a fungal disease of *Fraxinus* spp. caused by the Ascomycete *H. fraxineus*, and the native ash species *F. excelsior* is particularly susceptible. The phenomenon of ash dieback has been observed on continental Europe, probably since the mid 1990s although the cause was not established until sometime later. The pathogen was first properly described in 2006 under the name *Chalara fraxinea* (Kowalski 2006). Four years later it was determined that *C. fraxinea* was the asexual (anamorphic) stage of the fungus when, in accordance with convention, it was subsequently named after the sexual stage *Hymenoscyphus pseudoalbidus.* The name by which it should now be known is *H. fraxineus* (Baral et al. 2014).

Ash leaves are infected in late summer by germinating ascospores of *H. fraxineus,* which grow into the petioles, the rachises, and then the shoot or stem where they initiate lesions. Infected petioles and rachises die and fall to the ground where they overwinter to produce apothecia which release ascospores the following summer (Gross et al. 2012). Once the pathogen is in the apothecia / ascospore phase of its life cycle, inoculum builds up quickly and initiates numerous fresh infections. Where ash dieback has been present for more than ten years in countries without effective control measures, a large proportion of ash trees have become infected and eventually succumbed to this pathogen (Timmermann et al. 2011).

All-Ireland Chalara Control Strategy

H. fraxineus is not a regulated disease under the EU Plant Health Directive (Council Directive 2000/29/EC). When the pathogen was first identified in Ireland in late 2012 the respective official Plant Health Services in the Rep. Ireland and Northern Ireland recognised that this was an issue affecting the entire island and that there were no natural or other effective barriers to air-borne spread of the fungal pathogen. Hence the DAFM in Rep. Ireland and the DARD in Northern Ireland developed a single All-Ireland Chalara Control Strategy (Anon. 2013). This strategy was described in an inter-Departmental cross-border document, which was launched in July 2013 by the Minister of State at DAFM, Tom Hayes TD (Teachta Dála), and the DARD Minister Michelle O'Neill MLA (Member of the Legislative Assembly).

The control strategy provided a framework for the policy of identification, control and eradication of *H. fraxineus* in Ireland and set out the actions required. The main aims of the strategy were described as:

- *All incidents of ash dieback disease found on the island of Ireland are managed consistently and promptly in order to contain and eradicate the disease and to minimise the risk of the disease spreading and becoming established.*
- *A programme of work and development for an evidence base in the context of the International Plant Protection Convention's International Standard for Phytosanitary Measure ISPM No. 4, in order to make the case for the establishment of a Pest Free Area by the end of 2013 and seek consideration by the EU Standing Committee on Plant Health to recognise pest free status in EU legislation, through designation as a Protected Zone.*

The first key objective was to 'Reduce the risk of the disease becoming established in the wider environment'. *'Wider Environment Infection'* is defined as "*ash of any age, which is thought to have been infected by the spread of spores in the environment. This is distinct from disease outbreaks in 'recently planted' or 'new planting' which is thought to have been introduced onto a site by planting previously infected forest nursery plants*" (Anon. 2013). Evidence from other European countries had shown that once the pathogen is established in the wider environment it is impossible to control or even restrict its spread (Timmermann 2011). It was clear initially that all of the sites in Ireland where ash dieback was confirmed were relatively recently planted (< 6 years), i.e. there was a very high probability that the pathogen had been introduced on infected plant material. Hence as a first step, emergency legislation was introduced to control the importation of ash trees, seeds and wood. In October 2012 both ministers announced a 'Fortress Ireland' approach and restricted any importation of ash trees to the island (Anon. 2012a) and made amendments to the Wood and Bark Order governing the importation of ash wood (Anon. 2012b). A policy of eradication was also adopted by plant health authorities north and south. DARD and DAFM also assisted the authorities in Great Britain in developing the necessary Pest Risk Analysis for Britain and Ireland (Sansford 2013).

In Northern Ireland, up until late 2012, ash was an important component of many new government grant-aided mixed woodland areas, similar to the schemes operated in the Rep. Ireland. At the first record of ash dieback in Ireland, as a temporary measure (still in force), both DARD and DAFM suspended grant-aid for new ash plantings in forestry schemes. If this temporary measure was going to be successful in protecting the Irish ash population then it was necessary to eradicate the pathogen and be able to source disease-free trees for any future planting. In 2013 The National Roads Authority, Rep. Ireland suspended the use of ash in roadside plantings and now uses alternative species. Other actions have included raising public awareness through information meetings ongoing information updates on the Departments' websites, press releases, and national and local adverts and the development by DARD and DAFM of the Tree Check App for reporting ill-health in trees. In May 2014 an all-Ireland conference on the disease was jointly organised by DAFM and DARD in conjunction with the Agri-Food and Biosciences Institute (AfBi), Northern Ireland and the Society of Irish Plant Pathologists. This conference was very well attended and brought together presentations and discussions on the latest scientific knowledge on the disease and input from stakeholders and other interested parties. Training and advice continues to be provided on the biology of the disease and recognition of symptoms and on the procedures around the clearance of affected forest plantations or infected nursery stock.

First records of ash dieback in Ireland

The first confirmed record of ash dieback on the island of Ireland was in October 2012 in a young forestry plantation in County Leitrim, Rep. Ireland, which had been planted in 2009 with trees imported from continental Europe. The trees on this site, and on all ten other sites which had been planted with the same batch of imported trees, were subsequently destroyed under official supervision. Since that finding there have been a total of 195 confirmed findings in the Rep. Ireland (Table 2).

Table 2 Confirmed findings (as of 12th November 2015) of ash dieback disease (*Hymenoscyphus fraxineus*) in the Republic of Ireland (DAFM, 2015).

Location type	Confirmed findings Oct 2012 – Dec 2014	New findings in 2015 (12th Nov 2015)	Total (12 Nov. 2015)
Forestry plantations[1]	54	56	110
Horticultural nurseries	20	5	25
Garden Centres	4	0	4
Private gardens	7	0	7
Farm / Agri-environment plantings	23	2	25
Roadside plantings[2]	4	9	13
Hedgerows[3]	4	7	11
Total	116	79	195

[1] Refers to the total number of forestry plantations with confirmed findings.
[2] Refers to the number of counties with one or more confirmed findings in roadside plantings.
[3] Refers to the number of counties with one or more confirmed native hedgerow findings

The findings set out in Table 2 demonstrate the variety of settings in which infected ash trees have been found in the course of surveys conducted by DAFM inspectors. The vast majority of infected sites have now been cleared of ash plants. DAFM introduced a Reconstitution Scheme grant to support forest owners affected by the disease in the removal and destruction of ash trees and leaf litter and the reconstitution of the forest with alternative species to ash. To date, approximately 2 million ash trees have been removed and destroyed by deep burial or burning, with particular attention paid to the management of leaf litter because of its important role in the disease cycle. Where achievable, trees in an infected site were removed before flushing to reduce inoculum development and as far as possible material already on the ground was collected although it was recognised that it was very difficult to remove all potentially infective plant parts.

The most significant sites in terms of the epidemiology of the disease are those in the 'hedgerow' category. While the findings in all the other categories were clearly linked to imported planting material, incidence of the disease in hedgerows suggests probable spread of the disease in Rep. Ireland. In Northern Ireland the first finding of ash dieback was on plants in a recently established plantation in November 2012. Since then there have been 111 sites in which the pathogen has been confirmed (Table 3).

In almost every case the plantations affected were recently planted (< 6 years), and it was often possible to trace back to the original sources of the planting material. The sources were a limited number of nurseries outside Ireland and routinely the origins of the plants were on continental Europe. Having identified the source it was possible, with excellent co-operation from the nurseries involved, to trace forward to plantations established using plants from the same or similar batches of young trees. This intensive trace-backward / trace-forward surveillance led to identification of many new infected sites which were found early in 2013 and were destroyed soon thereafter.

Table 3 Confirmed findings (November 2015) of ash dieback disease (*Hymenoscyphus fraxineus*) in Northern Ireland (DARD, 2015).

Site Type	Age	Number of Outbreaks 2012/13 (Premises)	Number of Outbreaks 2014 (Premises)	Number of Outbreaks 2015 (Premises)	Total
Woodland Plantation	0-10	63	1	17	81
Urban/Amenity	0-5	9	0	1	10
Roadside	0-5	3	0	0	3
Private Garden	0-5	10	0	0	10
Hedgerow (recently planted)	0-5	3	1	0	4
Nursery Findings	0-5	3	0	0	3
Total		91	2	18	111

Systematic and targeted surveys

Following detection of the disease, plant health inspectors carried out intensive surveillance programmes. The initial approach to surveillance was one of 'trace-back / trace-forward'. Traceability mechanisms which are required under Council Directive 1999/105/EC on the marketing of forest reproductive material have enabled good 'trace forward' of imported forestry planting material, although similar traceability is not available in non-forestry sectors such as roadway plantings. Further to this, there were both structured surveys, i.e. to get maximum coverage of the whole country, and targeted buffer surveys, i.e. associated with outbreaks, which had been confirmed through laboratory tests.

DAFM has carried out intensive surveys for the disease since the first finding in 2012. The surveys have been carried out in line with ISPM No. 6 (Anon. 2005) incorporating systematic and targeted, risk-based components. Targeted forestry surveys have involved inspections of hundreds of plantations where imported ash plants had been planted. Where infected ash plantations have been cleared, follow up surveys have been carried out in subsequent years to identify any evidence of spread from the original infected sites to the surrounding environment.

In the Rep. Ireland the systematic survey element was based on a 2 x 2 km grid. Approximately 370 sites (both National Forest Inventory plots and hedgerow points) were surveyed. To date the results of the systematic grid-based survey have been negative. In addition to these elements, DAFM follows up on reports of suspicious ill-health of trees from stakeholders.

In both the Rep. Ireland and Northern Ireland many of the new plantations that have been planted in the recent past have been supported by Government planting schemes. Using this information, further plantations were identified which were surveyed and on some occasions were found to be infected. It was more difficult to identify where ash had been planted as part of a landscaping programme, e.g. new roadsides, as these were usually not grant-aided and therefore details of sources of plants were not always available. Such sites were often only identified by inspectors on an *ad hoc* basis.

In Northern Ireland, at every site where ash dieback was identified, a standard 500 m buffer survey was carried out. At sites where there was evidence of spread within or without the plantation an intensive survey was conducted on all hedgerows including both roadside hedges and field boundaries within a 250 m radius. Any plants with dieback symptoms were submitted to the laboratory for diagnosis. A less intensive survey was conducted out to a distance of 500 m. In 2015 (until November) alone, in total, DARD inspectors conducted over 1,800 surveys (Figure 1A).

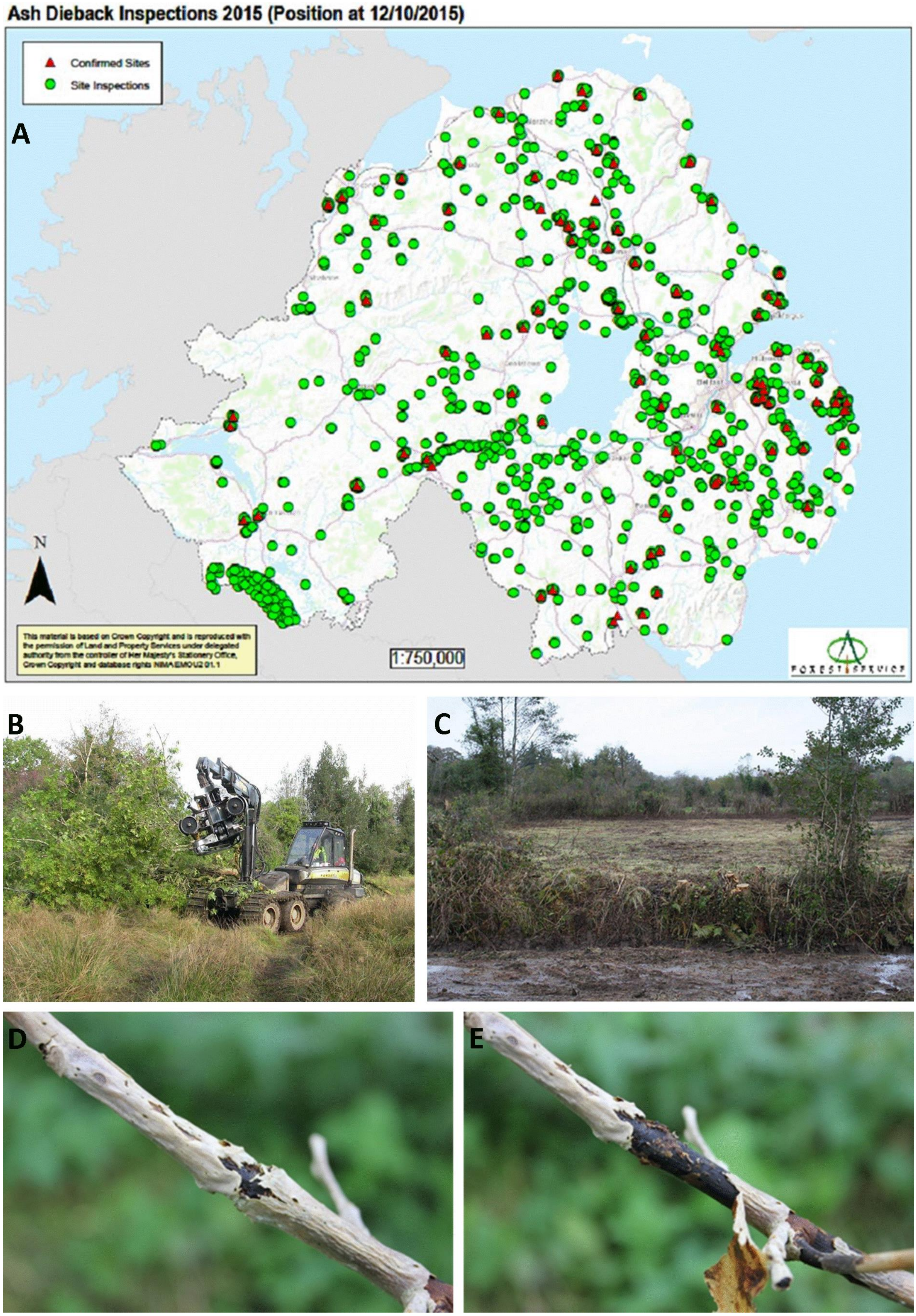

Figure 1 (see next page for descriptions)

Figure 1A) Northern Ireland plant health inspections of ash plantings where ash dieback could be present (these include buffer surveys of areas associated with previous outbreaks; trace-forward / trace-back plantings; *ad hoc* findings; structured surveys) carried out January – November 2015. Green dots were survey sites where ash dieback was not detected. Red triangles were sites where symptoms of ash dieback were observed and the pathogen *H. fraxineus* was detected using laboratory tests. **1B)** Harvester at work at the Leitrim site (Coillte, DAFM). **1C)** Cleared field post eradication works (Forest Service, DAFM). **1D-1E)**Woody tissue still attached to dead tree with papery epidermis and remains of apothecia below the epidermis.

On the confirmation of the presence of the pathogen on a site a Statutory Plant Health Notice was issued preventing the movement of plants from the site, imposing bio-security measures and prohibiting further planting of ash at the site and requiring the removal and destruction of all ash plants and associated plant debris.

Detection of *Hymenoscyphus fraxineus*

Inspectors collected samples of ash trees with dieback symptoms. Great care was taken to avoid cross-contamination between samples because of the sensitivity of the detection methods, particularly those based on DNA analysis. Inspectors wore fresh disposable nitrile gloves for every sample, equipment was flame-sterilised between samples and samples were bagged separately, along with the gloves used, and sealed. Before leaving a site inspectors used a biocide to spray boots and clothes to avoid potential movement of the pathogen to disease-free sites. Samples were submitted to either the Agri-Food & Biosciences Institute Laboratories in Belfast or to the DAFM Laboratories at Backweston, Co. Kildare. Routinely, DNA was extracted from samples and subjected to validated Real-Time PCR testing using *H. fraxineus* specific probes and primers (Ioos et al. 2009; Ioos and Fourrier 2011). This enabled large numbers of samples to be processed rapidly and results were very consistent. This methodology is highly sensitive giving C_t (cycle threshold) values in the range 18 – 32. On occasions where low-level positives (C_t values > 32) were recorded a fresh sample was collected and sent to the laboratory for analysis.

Routinely, but not with every sample submitted, an attempt was made to isolate the pathogen. The best success was obtained when isolating from fresh, large, well-developed stem lesions, onto Potato Dextrose Agar (PDA) plates. After placing the piece of plant tissue on the agar, the plates were incubated for up to four weeks at 4° C before transferring them to 18° C after some growth of *H. fraxineus* had developed. Isolation was obviously of limited value for routine processing of samples but did enable laboratories to build up a culture collection of isolates obtained from trees growing in Ireland. It was also useful in confirming that the pathogen was viable and growing actively.

When ash dieback was confirmed at a site, a search was made for infected leaf rachises and petioles with or without apothecia. This was to indicate whether or not the pathogen had entered the epidemic phase of the life cycle. In all but the most heavily affected sites, few apothecia were found in the field. On several occasions apothecia did develop after a few weeks' incubation of plant material under high humidity in the laboratory at room temperature in the light. Apothecia were subjected to molecular analysis to confirm that they were *H. fraxineus* (*pseudoalbidus*) and not *H. albidus.* The latter was not detected in any routine samples but was confirmed by AFBI analyses in samples collected by DAFM in samples from Rep , Ireland, counties Cavan (May 2015) and Leitrim (June 2015).

Furthermore, *H. albidus* was found as ascocarps on ash petioles in the leaf litter of a 10-year-old gene bank of ash trees in Dublin in September 2015. It was notable that the fructifying rachises were found in the first and second row of trees near the edge of the plantation. Typical asci and spores were observed using light microscopy (Figure 2) and the absence of a characteristic crozier at the base of the asci was noted. The crozier feature is a characteristic feature *H. pseudoalbidus* (*fraxineus*) (Zhao et al. 2012). DNA was extracted from the ascocarp tissue and was confirmed as *H. albidus* by real- time PCR using the TaqMan method capable of specifically detecting *H. albidus* described in Husson et al. 2011. These were the first recorded confirmation of *H. albidus* in Ireland.

Eradication

In Northern Ireland when *H. fraxineus* was detected on a site all of the ash trees within that plantation were destroyed. If the trees were very small it was usually possible to uproot them. Larger trees were cut as close to ground level as possible. The cut stump was treated with glyphosate herbicide to prevent re-growth. These sites were checked the following year to ensure that all trees had been killed. The cut trees were deep buried, burned *in situ*, or removed to a landfill site. On bigger plantations and with larger trees they were chipped, under cover, into a covered trailer and the chips put in a landfill site or burned *in situ*. The cost of removal was borne by the Government Forest Service and the landowners were offered freely a selection of tree species to re-establish their woodlands. As far as was practically possible, great care was taken to remove as much as possible of the plant debris on the ground. If trees could be removed before autumn leaf fall this was considered highly desirable. A similar protocol was followed in the Rep. Ireland.

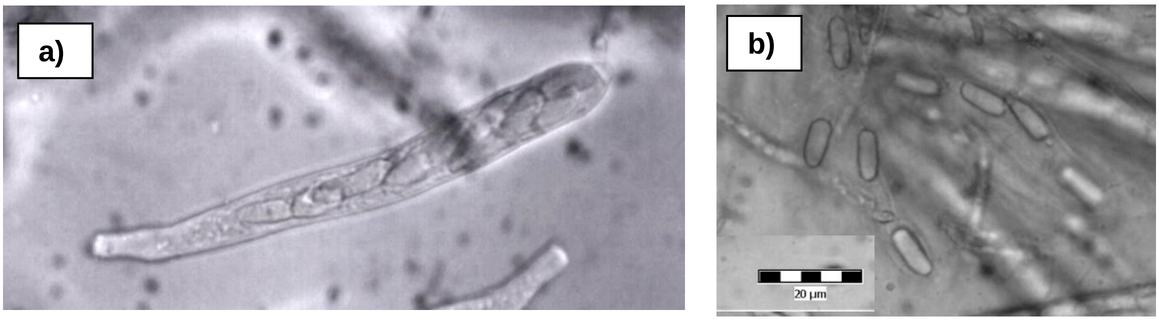

Figure 2 a) Ascus and **b)** spores of *H. albidus* from an Irish source

Case study 1: Co. Leitrim, Republic of Ireland

As mentioned previously, the first case of ash dieback in Ireland was confirmed in October 2012 in a young forestry plantation in County Leitrim (N: 54° 05'; W: 7° 48'), which had been established in 2009 with *F. excelsior* trees imported from continental Europe. The Leitrim forest plantation was 3.85 ha of which 2.3 ha was ash. All planted ash trees (5,700) at the site were cut, the stumps treated with herbicide and the cut trees and leaf litter burnt on site in October 2012. At the time of clearance, the trees were c. 2 m in height and approximately 50% of the planted ash showed symptoms of the disease. Ash was plentiful in internal hedgerows on the site and in the vicinity of the site but at this stage symptoms were not observed in the hedgerows.

Surveys in the 2013 growing season, however, led to confirmation of the presence of the disease in a number of hedgerow trees within and beside the original infected plantation site. At the time this was the first site on the island where the evidence suggested spread of the disease from imported material to wild ash trees. Following demarcation surveys, a major eradication effort was then undertaken at this site commencing in October 2013 with the aim of removing all host material and leaf litter. A 250 m buffer zone was demarcated around the plantation, which incorporated all the known positive trees. This resulted in a 60 ha demarcated zone and contained approximately 12 km of hedgerows of which 7.5 km contained ash. All host material within the demarcated area was removed and destroyed. Tree clearance involved a commercial timber harvester (Figure 1B), a forwarder and a team of three chainsaw operators.

Stumps of cut trees were treated with herbicide. An excavator was used to gather branch material and leaf litter, including clearing drains of material, for burning on site (Figure 1C). In total 850 ash trees >10 cm dbh and 850 ash trees <10 cm dbh were felled and destroyed.

In early 2014 further clearance operations were carried out to eliminate any material that was missed and any re-growth within the demarcated zone was cut and sprayed with herbicide. Follow- up surveys were carried out in the wider area. This led to the detection of a number of infected trees outside the demarcated area up to approximately 2 km from the original infected site. In 2015, surveys led to further findings of the disease at increased distance (over 10 km) from the original site.

Case study 2: Co. Antrim, Northern Ireland

In August 2015 in Co. Antrim a small mixed plantation including ash was found to have a high level of *H. fraxineus* infection. The trees had been planted in 2008 using imported ash saplings from a nursery in Great Britain. On initial survey of the site a high proportion of trees was displaying some level of infection. Symptoms of ash dieback on infected trees were classified into broad groups in order to postulate a disease time-line:

- Infected at planting: These trees were small and many had died within the first one to three years of planting. Often the leaders had died and there was evidence of shoot production from the base of the plants.
- Side shoot infections: Side shoots and small branches were dead due to infections which had occurred through leaves and petioles. By assessing the extent and size of the shoots and associated lesions it was possible to estimate the year that leaf infection have occurred.

- Basal lesions: Large lesions occurring at the base of the trees at soil level. These lesions were not associated with any stem infections higher up. It was postulated that rachises and petioles trapped in grass had produced large numbers of apothecia and copious amounts of ascospores which infected the base of the trees. Lesions near the base of the trees could have been the result of mechanical damage during uprooting in the nursery. Several of these lesions were confirmed by molecular methods to be infected with *H. fraxineus*.

- Stem lesions: Characteristic diamond-shaped lesions which appeared to have developed from a side shoot infection rather than on a plant which had been infected prior to planting.

Leaf symptoms, spotting, vein and petiole lesions and wilting, which had occurred during the growing season 2015 apparently emanating from apothecia formed as a result of 2014 leaf infections and / or from other airborne incursions.

It was clearly evident that the pathogen had been, and still was, actively circulating within the plantation. This raised the question as to how inoculum had built up sufficiently. When planted, the trees were leafless, two-year-old saplings with no obvious symptoms, and yet infected rachises were found producing sporulating apothecia on the plantation floor. It was considered unlikely that the pathogen had grown from the stem lesion back into the rachises or petioles of uninfected shoots.

Initial development of apothecia

In the life cycle proposed by Gross et al. (2012) apothecial development occurs on fallen petioles and rachises with ascospore release during the summer months. For this to occur it is necessary to have had infection initially on the leaves. Once this occurs, then the life cycle can continue and the inoculum can build up exponentially. However, the trees, which were planted in Ireland and subsequently developed ash dieback symptoms on the stems at all the sites, had been imported as small (generally two-year-old) leafless saplings. It can be assumed that symptoms were not obvious at the time of planting, since such plants would have been rejected. Since no leaves were present on the saplings there would have been no petioles or rachises on which apothecia could form as described by Gross et al. (2012). This therefore raised the question about how the pathogen moved from a lesion on the stem to colonise petioles and rachises, which in turn can develop apothecia.

A number of hypotheses were considered.

1. Introduction of the pathogen on leaf and rachises debris

When plants are bundled in the nursery for transportation for planting it is inevitable that there is a certain amount of plant debris and leaves associated with them. If the plants were coming from an infected nursery it may not be surprising that some pathogen inoculum would be introduced in this way. Infected rachices may harbour the pseudosclerotial stage of Chalara, for one or more years before optimal conditions prevail to favour the onset of ascospore production and so they have the potential to become inoculum sources over time. It is relatively unlikely that a nursery would be heavily infected and continue commercial production of ash plantssince, apart from other considerations, this would be uneconomic, therefore only low levels of inoculum would be introduced by this route and this would take a considerable time to initiate significant amounts of infection.

2. Movement of the pathogen out of the stem lesion back along a petiole to cause a leaf infection.

This was thought to be unlikely and no evidence was ever observed of the pathogen moving from the stem into the petiole.

3. Development of apothecia on roots.

This was considered not impossible and the likelihood of it occurring has been discussed during FRAXBACK meetings. At the Co. Antrim site, on the roots of many of the dead young trees which were examined, pseudosclerotia were observed and on a small number of these apothecial formation was induced following incubation in the laboratory. The development of apothecia on infected roots has been thought to be a very rare occurrence and this is the first report for Ireland. Nevertheless the experience of this site would suggest that under suitable conditions apothecia can form on roots readily. They seemed to occur more commonly on roots from trees which had been growing in water-soaked soil.

4. Development of apothecia on woody tissue

Several trees on the site had been dead for some time. The epidermis on their shoots and small branches had become pale and papery in appearance. This was easily peeled away and on several occasions fully developed apothecia were found growing on the wood (Figure 1D and 1E). This occurred at a height of up to 3 m on many trees. While ascospores were unlikely to escape from beneath the dried epidermis while the branches were *in situ,* when these branches fell to the ground, apothecia could be exposed and release their spores. The spread of the pathogen within the plantation was relatively rapid at sites where there was a significant initial level of tree death. If large numbers of trees were dying in the first year or two after planting then the potential for significant inoculum to build up on the woody tissues was high. This is therefore considered to be possibly an important source of inoculum development.

The potential development of apothecia on shoots and roots is of significantly greater importance in Ireland than other countries where aerial inoculum is the primary means of dissemination. The Irish maritime climate and in particular wet soils may also influence the formation of apothecia on these plant tissues. The period of ascospore production would appear to be extended into the autumn and the lack of severe winter temperatures could encourage pathogen survival.

Spore sampling

Four Burkard® spore samplers were employed at a range of sites across Northern Ireland in 2014 and 2015. In both years between May and October a sampler was sited at Glenwherry Hill Farm (NW 397 567). The Hill Farm is on the Antrim Plateau in the east of Northern Ireland, where there were almost no ash plants in the landscape. However, the mathematical models developed by the University of Cambridge, incorporating data supplied by DAFM and DARD, indicated that if *H. fraxineus* ascospores were airborne, particularly if originating from Great Britain or the continent, this would be the most likely area in which they would be deposited (Matt Castle; personal communication). No ascospores were detected. In 2014 two samplers were placed in a positive ash dieback site in Co. Tyrone (NV 564 142) from which all ash trees had been removed the previous winter. No ascospores were detected. In 2015 a sampler was run from May to October at a positive site in Co. Londonderry (NW 101 702). At this site, infected plants had been removed in 2013, but a small clump of larger trees had been missed and was not removed until late 2014. There was some evidence of apothecial development on fallen rachises. By mid September small numbers of spores were observed and in one week only in late September, coinciding with a period of warm dry weather, there was significant release of spores. Thereafter, only small numbers of spores were observed. A sampler was run from July to November in the far west of Northern Ireland in Co. Fermanagh (NV 213 003) close to the border with the Rep. Ireland and approximately 20 km from the site described in Case Study 1. While the running of the sampler was somewhat inconsistent, no ascospores were detected during the summer. The fourth sampler was placed at the Co. Antrim site, Case Study 2 (NW 206 518) in mid August. Throughout the summer infected rachises were commonly found. Very few spores were detected during August. However in mid September there was evidence of profuse spore production similar to that found at the Co. Londonderry site. While this reduced significantly after that, spores were still being observed up until early November.

In summer 2015, DAFM conducted a sporetrapping experiment around two infected sites in collaboration with and using a methodology developed by INRA, France’s National Institute for Agricultural Research. The experiment aimed to detect, quantify, and establish dispersal patterns around these known positive locations. Results are still being analysed.

Potential to identify ash dieback resistant ash

The genetic diversity of Irish ash populations was analysed using neutral markers and this has shown that 96% of the variation is contained within the population with little evidence of population structuring (Thomasset 2011; Thomasset et al. 2013). This apparent lack of genetic diversity compares with results from other countries (Heuertz et al. 2001; Morand et al. 2002; Fernández-Manjarrés et al. 2006; Ferrazzini et al. 2007). This means that it will be unlikely to identify distinct populations of dieback resistant trees, since the probability of sampling differentiated individuals within a population is similar to the probability of sampling differentiated individuals in two different populations, because of an almost random distribution of genetic diversity within and among populations. Molecular markers were also used in efforts to characterise hybrids of ash which were imported to Ireland in the period 1990-2002. The material was shown to consist of hybrids of *F. excelsior* with *F. angustifolia.* In many cases, imported trees were mal-adapted showing poor growth and stem form in plantations (Douglas and Thomasset 2014; Thomasset et al 2011b). The percentage of hybrid trees detected in two intensively sampled plantations ranged from 28 - 58%. These imports were referred to as cryptic hybrids because of a lack of intermediate morphological features which are common in pure F1 hybrids (Thomasset et al. 2013; Thomasset et al 2011a). Some hybrid trees still remain after thinning, however their susceptibility to *H. fraxineus* is not known. Efforts to find Irish genotypes of ash with tolerance to *H. fraxineus* are underway by Teagasc, the Rep. Ireland agricultural science research organisation. Irish genotypes of ash were propagated vegetatively in 2013 and planted out in test environments with high disease pressure in 2014 (France and Lithuania). At the end of the first season of growth, evidence for infection had already been found among the trees planted in Lithuania indicating good prospects for finding *H. fraxineus* tolerant genotypes by this approach.

DAFM is also participating in a five-year project which was begun in 2013, the aim of which has been to produce individual trees of ash which show resistance or tolerance to Ash Dieback and to use them to bulk up stocks of resistant trees as well as to establish seed-producing orchards with resistant parent trees. The project, which is part funded by DAFM, is being carried out by Forest Research, an agency of the Forestry Commission in the UK. The project involves 48 hectares of trial plantings over fourteen sites in the east of England and the mass screening of some 155,000 ash trees with fifteen different provenances from continental Europe, the UK and Ireland. Over 14,000 Irish ash plants from two distinct seed lots are included in the trials.

Conclusions

Protection of the plant health status of Ireland is a high priority. It is clear that ash dieback was brought into the island of Ireland on infected plants for planting. This emphasises that growers have a high degree of responsibility towards their customers to produce, and trade in disease-free plants. Similarly, landowners should become more fully aware of the materials they deploy in forests and gardens: i.e. the provenance of the seeds / saplings, the locations of sapling production and the traceability systems in operation. Local nurseries and producers should take up opportunities to grow native plants for local usage and customers should be prepared to meet cost differentials occasioned by, for example, lack of economies of scale in local nurseries.

There may be an argument that Government could help with facilitating more sustained local sapling production by forward planning of grant-aided forestry Schemes, since it is in its long-term interest. Sustained forward planning is also in the interest of nurseries because they require at least three years notice to supply plants for any new grant scheme which will potentially increase the demand for trees. To date the interventions undertaken by the respective Government Departments have had a major impact in mitigating the establishment of ash dieback in Ireland and in attenuating its spread to the wider environment. *H. fraxineus* is, however, a highly infective pathogen and it is therefore very difficult to predict if it will be possible to prevent the further spread of ash dieback within Ireland.

There is reasonable confidence that no new inoculum of *H. fraxineus* has been introduced into the island through trade since the ban on importation of ash plants for planting, although there is the risk of some importation occurring through unregulated trade. The policy restricting the movement of ash plants into Ireland may have to change in the near future, in line with general EU legislation, which will potentially increase the risk of future introductions. Current epidemiological modelling has suggested that ingress of ascospores from Great Britain or Europe in numbers sufficient to incite an epidemic is unlikely. This situation may change as the sources and quantity of inoculum production increases throughout the UK and Europe. Despite findings of the disease in a total of over 300 sites or holdings on the island of Ireland, these have consisted almost exclusively of material planted within the last six years. However, there are a few places on the whole island where it could be considered that the pathogen has spread to trees in the hedgerow environment.

In both the Rep. Ireland and Northern Ireland there have been a few locations where the pathogen has been detected in isolated trees or hedges with no obvious link to infected sites. These anomalous cases may be explained by ingress of aerial spores and / or by spore production from infected trees from unknown sources in nearby landscape plantings. However the findings of infections in hedgerows associated with diseased plantations illustrate the highly infective nature of the pathogen, These localised infections probably originated from ascospores produced on small quantities of leaf debris and / or necrotic shoots and roots. While it is known that the pathogen can spread very rapidly across regions where there is an adequate aerial inoculum, the authors consider that the current situation in Ireland is of a slow rate of spread (based on survey data). Continued intensive surveying and monitoring of positive sites, together with data from spore trapping, will provide evidence for the likelihood of success of the current disease eradication policy. Removal and destruction of infected ash trees and leaf debris at infected plantations will contribute to reducing the build up of inoculum and slowing the spread of the disease.

There are over 800 risk entries on the Department of Environment, Food and Rural Affairs (DEFRA) Plant Health Risk Register (Anon. 2015b). Many of these are potential high plant health risks to agricultural, horticultural, forestry and amenity crops and plants in Ireland. It is therefore essential to engage in horizon-scanning in order to identify threats at an early stage. Furthermore, because of the constant issue of emerging diseases and pests, a more rigorous system of traceability is now advised for all categories of imported trees and shrubs throughout Europe. Consideration also needs to be given as to how pests and pathogens can more effectively be detected at points of importation.

Ash is a high profile species. The disease has led to increased awareness among the public and industry of the importance of plant health and the potential negative implications of trade and non-trade/other transfers of plant material on plant health status. This provides an opportunity to develop greater future public engagement with plant health, e.g. by using the Tree Check app for wider plant health monitoring. The latest figures from the Republic of Ireland are that 733 hectares of ash plantation has been reconstituted with another species as a result of Chalara and this has cost our state €2.6 million so far; in addition, Chalara has been found and confirmed in all 26 counties of the Republic of Ireland to a greater or lesser extent. As a result the current policies and procedures regarding Chalara are under review.

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References

Anon. 2005. International Standards for phytosanitary measures; ISPM No. 6. Guidelines for Surveillance (1997). Secretariat of the International Plant Protection Convention (FAO, 2005). http://www.acfs.go.th/sps/downloads/13717_ISPM_6_E.pdf

Anon. 2012a. Fortress Ireland approach to prevent spread of ash disease essential. http://www.northernireland.gov.uk/index/media-centre/news-departments/news-dard/news-dard-october-2012/news-dard-261012-fortress-ireland-approach.htm (accessed 23rd November 2015).

Anon. 2012b. Plant Health (Wood and Bark) (Amendment) Order (Northern Ireland) 2012. http://www.legislation.gov.uk/nisr/2012/400/article/2/made (accessed 23rd November 2015).

Anon. 2013. All-Ireland Chalara Control Strategy. https://www.dardni.gov.uk/publications/all-ireland-chalara-control-strategy (accessed 23rd November 2015).

Anon. 2015a. Hedgerows. Notice Nature Ireland http://www.noticenature.ie/files/hedgerows.pdf (accessed 19th October 2015).

Anon. 2015b. UK Plant Health Risk Register: Department for Environment, Food & Rural Affairs. https://secure.fera.defra.gov.uk/phiw/riskRegister/ (accessed 10 December 2015).

Anon. 2015c. Heritage Council of Ireland 2015. New Initiatives Launched to Safeguard Ireland's Unique Hedgerows - accessed 20 November 2015). County surveys: See *http://www.heritagecouncil.ie/* (accessed 20th November 2015).

Baral, H-O, Queloz, V and Hosoya, T. 2014. *Hymenoscyphus fraxineus*, the correct scientific name for the fungus causing ash dieback in Europe. *IMA Fungus* 5: 79 - 80

Black, K., Green, S., Mullooly, G. and Povida, A., 2014. Towards a national hedgerow biomass inventory for the LULUCF sector using LiDAR remote sensing (2010-CCRP-DS-1.1): Final report, EPA, Dublin. https://www.epa.ie/pubs/reports/research/climate/ccrp-32-for-webFINAL.pdf

Brasier, C. M. 2008. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* 57: 792 – 808.

DAFM, 2015. Ash dieback (Chalara) http://www.agriculture.gov.ie/forestservice/ashdiebackchalara/ (accessed 20th November 2015).

Drenkhan, R., Sander, H. and Hanso, M. 2014. Introduction of Mandshurian ash (Fraxinus mandshurica Rupr.) to Estonia: Is it related to the current epidemic on European ash (F-excelsior L.)? *European Journal of Forest Research* 133: 769 – 781.

Dobrowolska, D., Hein, S., Ossterbaan, A., Wagner, S., Clark, J. and Skovsgaard, JP. 2011. A review of European ash (*Fraxinus excelsior* L.) implications for silviculture. *Forestry* 84: 133 – 148.

Douglas, G. C. and Thomasset M. 2014, Identifying and characterising hybrid 'brown bud' ash in Ireland. *COFORD Connects- Reproductive material* No. 20, 1-6.

Fernández-Manjarrés, J. F., Gerard, P. R., Dufour, J., Raquin, C. and Franscaria-Lacoste, N. 2006. Differential patterns of morphological and molecular hybridization between *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl (Oleaceae) in eastern and western France. *Molecular Ecology* 15: 3245 – 3257.

Ferrazzini, D., Monteleone, I. and Belletti, P. 2007. Genetic variability and divergence among Italian populations of common ash (Fraxinus excelsior L.). *Annals of Forest Science* 64: 159 – 168.

Foulkes, N. .2006. County Leitrim hedgerow survey report. *An Chomhairle Oidhreachta / The Heritage Council* 2006. pp 92

Green, S., 2010 The Irish hedge map – version 1.0 Teagasc Technology Update www.teagasc.ie (accessed 20th November 2015).

Gross, A., Zaffarano, P. L., Duo, A. And Grunig, C. R. 2012. Reproductive mode and life cycle of the ash dieback pathogen. *Fungal Genetics and Biology* 49: 977–986.

Heurtz, M., Hausman, J-F., Tsvetkov, I., Franscaria-Lacoste, N. and Vekemans, X. 2001. Assessment of genetic structure within and among Bulgarian populations of the common ash (*Fraxinus excelsior* L.). *Molecular Ecology* 10: 1615 – 1623.

Husson, C., Scala, B., Cael, O., Frey, P., Feau, N., Ioos, R. and Marcais, B. 2011 *Chalara fraxinea* is an invasive pathogen in France. *European Journal of Plant Pathology*,130 (3): 311-324.

Lawrence, R. and Cheffings, C.M. 2014. A summary of the impacts of ash dieback on UK biodiversity, including the potential for long-term monitoring and further research on management scenarios. *Joint Nature Conservation Committee, Report No. 501.* Peterborough 2014.

Ioos, R. and Fourrier, C. 2011. Validation and accreditation of a duplex real-time PCR test for reliable *in planta* detection of *Chalara fraxinea*. *OEPP/EPPO Bulletin* 41: 21 – 26.

Ioos, R., Kowalski, T., Husson, C. and Holdenrieder, O. 2009. Rapid *in planta* detection of *Chalara fraxinea* by a real-time PCR assay using a dual-labelled probe. . *European Journal of Plant Pathology* 125: 329 – 335.

Kowlaski. T. 2006. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxineus excelsior*) in Poland. *Forest Pathology* 36: 264 – 270.

McCann, T. 2012. The Woody Species Diversity of Hedges in Relation to Environment, Landscape, History, Management and Structure in Northern Ireland. PhD Thesis, Queen's University of Belfast 2012.

Mitchell, R.J., Bailey, S., Beaton, J.K., Bellamy, P.E., Brooker, R.W., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Farren, J., Gimona, A., Goldberg, E., Hall, J., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Hooper, R.J., Howe, L., Iason, G.R., Kerr, G., Littlewood, N.A., Morgan, V., Newey, S., Potts, J.M., Pozsgai, G., Ray, D., Sim, D.A., Stockan, J.A., Taylor, A.F.S. & Woodward, S. 2014. The potential ecological impact of ash dieback in the UK. *Joint Nature Conservation Committee, Report No. 483.* Peterborough 2014.

Morand, M-E., Brachet, S. Rossignol, P. and Dufour, J. 2002. A generalized heterozygote deficiency assessed with microsatellites in French common ash populations. Molecular Ecology 11: 377 – 385.

Sansford, C. E. 2013. Pest Risk Analysis for *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*) for the UK and the Republic of Ireland. Forestry Commission. May 24th 2013. 128pp.
https://secure.fera.gov.uk/phi/riskRegister/plant-health/documents/hymenoscyphusPseudoalbidusPRA.pdf (accessed 20th November 2015).

Thomasset, M. 2011 Introduced hybrid ash: *Fraxinus excelsior* X *F. angustifolia* in Ireland and its potential for interbreeding with native ash. Ph D. Thesis (2011), University of Dublin Trinity College, Ireland.

Thomasset, M., Fernández-Manjarrés, J. F., Douglas, G. C., Bertolino, P. Frascaria-Lacoste, N. and Hodkinson, T. R. 2013. Assignment testing reveals multiple introduced source populations including potential ash hybrids (*Fraxinus excelsior* X *F. angustifolia*) in Ireland. *European Journal of Forest Research* 132: 195-209. http://rd.springer.com/article/10.1007%2Fs10342-012-0667-9.

Thomasset M., Fernández-Manjarrés, J.F., Douglas G.C., Frascaria-Lacoste, N., Raquin, C. and Hodkinson, T.R. 2011a. Molecular and morphological characterization of reciprocal F_1 hybrid ash (*Fraxinus excelsior F. angustifolia*, Oleaceae) and parental species reveals asymmetric character inheritance. *International Journal of Plant Sciences* 17: 423-433. Stable URL: http://www.jstor.org/stable/10.1086/658169.

Thomasset,M., Fernández-Manjarrés, J.F., Douglas, G.C., Frascaria-Lacoste, N. and Hodkinson, T.R. 2011b. Hybridisation, introgression and climate change: a case study for the tree genus *Fraxinus* (Oleaceae). In: *Climate Change, Ecology and Systematics*, ed. Trevor R. Hodkinson, Michael B. Jones, Stephen Waldren and John A. N. Parnell. Published by Cambridge University Press© The Systematics Association 2011 pp. 320-342.

Timmermann, V., Borja, I., Hietala, A. M., Kirisits, T. and Solheim, H. 2011. Ash dieback: pathogen spread and diurnal patterns of ascospore dispersal with special emphasis on Norway. Bulletin OEPP/EPPO 41: 14 - 20

Zhao Yan-Jie , Tsuyoshi Hosoya, Hans-Otto Baral, Kentaro Hosaka & Makoto Kakishima 2012. *Hymenoscyphus pseudoalbidus*, the correct name for *Lambertella albida* reported from Japan. *Mycotaxon* 122: 25-41.