Received: 13 April 2022 | Revised: 5 December 2022 | Accepted: 12 December 2022

DOI: 10.1111/efp.12789

Forest Pathology WILEY-BLACKWELL WILEY

ORIGINAL ARTICLE

The introduction of *Hymenoscyphus fraxineus* to Northern Ireland and the subsequent development of ash dieback

Emma Baxter[1] | Louise R. Cooke[1] | Florentine Spaans[2] | Irene R. Grant[1] | Alistair R. McCracken[2]

[1]School of Biological Sciences, Queen's University of Belfast, Belfast, UK

[2]Agri-Food & Biosciences Institute, Belfast, UK

Correspondence
Irene R. Grant, School of Biological Sciences, Queen's University of Belfast, 19 Chlorine Gardens, Belfast BT9 5DL, UK.
Email: i.grant@qub.ac.uk

Funding information
Northern Ireland Department of Agriculture, Environment and Rural Affairs PhD Studentship

Abstract

Ash dieback caused by *Hymenoscyphus fraxineus* was first recorded in Northern Ireland (NI) in November 2012. The disease was observed only on recently (<6 years) planted trees. An in-depth case study in 2015 of an ash plantation with severe symptoms indicated that many of the trees were infected at the time of planting. Apothecia were observed developing from pseudosclerotia beneath the epidermis of dead branches still attached to the tree, suggesting a possible mechanism whereby *H. fraxineus* could be disseminated without leaf or rachises infection. Apothecia also formed on roots, indicating that infections may also occur in the soil. Often young trees were killed by the formation of large basal lesions which did not arise from stem infections higher up. On first detecting the disease on the island of Ireland the Governments of NI and the Republic of Ireland published an "All-Ireland Chalara Control Strategy." Part of that strategy was a ban on the importation of ash plants from regions where the disease was known to be present, to prevent the introduction of further inoculum, and the implementation of an 'eradication and containment' policy with the aim of preventing the establishment and spread of the disease. While these measures may have slowed disease establishment, they were ultimately unsuccessful and by 2018 ash dieback was widespread and established throughout the whole of NI in plantations and in the wider environment.

KEYWORDS
apothecia, ash dieback, eradication and containment, *Hymenoscyphus fraxineus*

1 | INTRODUCTION

Ash dieback is a devastating disease of European or common ash (*Fraxinus excelsior*). Other species of ash are susceptible but to a lesser extent (Drenkhan & Hanso, 2010; Kirisits et al., 2009). Recently, ash dieback was reported on 23 *Fraxinus* taxa growing in Slovak arboreta (Pastirčaková et al., 2020). The causal pathogen is now known as *Hymenoscyphus fraxineus* although when first identified it was named *Chalara fraxinea* (Kowalski, 2006). A full account of the naming of this organism is given by Baral et al. (2014). In Europe, the symptoms of ash dieback were first observed in Poland in 1992 (Przybyl, 2002). *H. fraxineus* is an Ascomycete fungus that forms

Alistair R. McCracken: Retired.

apothecia on the rachises of fallen ash leaves from which ascospores are released in the late summer and early autumn (Timmermann et al., 2011), and these are the primary source of infection. The disease has since spread rapidly westward and northward so that it is now present, and in most cases well-established, in many countries from Eastern Europe, Scandinavia and the British Isles (EPPO, 2022). In every country where ash dieback has become established, it has had a significant impact on the ash population, infecting forest trees, hedgerows and landscape plants of all ages and stages of development (e.g Cleary et al., 2017; Heinze et al., 2017; Rozsypálek et al., 2017).

Introduction of *H. fraxineus* to new regions and the subsequent dissemination of the pathogen is primarily through air-borne ascospores produced in the summer in apothecia on the previous year's leaf remnants (Timmermann et al., 2011). Chandelier et al. (2014) found that most ascospores were deposited downwind within 50 m of an infection with only 0.6%–0.7% of the inoculum density reaching that far. Grosdidier et al. (2018) tracked the dispersal of *H. fraxineus* airborne inoculum. They estimated that the mean distances of dispersal, either local or regional, were 1.4 and 2.6 km, respectively. However, they also found that spores were detected up to 50–100 km ahead of the disease front, which proved the presence of the pathogen before observations of the symptoms. Where countries, including Norway, Switzerland, France and Italy, tracked the concentric expansion of the disease they typically found continuous steps of about 30–70 km/year (Enderle et al., 2019). Using Lagrangian Stochastic Models (MASAD), Yearsley (2016) indicated that ash dieback spores could have been deposited along the eastern part of Northern Ireland (NI) or Republic of Ireland (RoI). However, it is considered by the authors of the current paper that it was highly unlikely that *H. fraxineus* initially arrived in Ireland through air-borne inoculum originating in Great Britain (McCracken et al., 2017). Ash dieback quickly became widely established in NI and the possible introduction of additional airborne inoculum from Great Britain was no longer a concern.

The island of Ireland (encompassing two jurisdictions: the ROI and NI part of the United Kingdom of Great Britain and Northern Ireland) is an island to the north west of continental Europe. Its geographic position confers a strong plant health status, and it had been hoped that the island of Ireland could be kept free of ash dieback. In October 2012, the disease was confirmed in a mixed tree planting in Co. Leitrim, ROI (McCracken et al., 2017). The trees in question had been planted 5–6 years previously. Despite intensive searching at that time, no symptoms of the disease were detected in the immediate (500 m radius) wider environment. A few weeks later, symptoms of ash dieback were found on a small number of young ash trees at a recently established plantation in Co. Antrim, NI, and the presence of *H. fraxineus* is confirmed (McCracken et al., 2017). Over the next 3 years, intensive structured surveys found the disease at many sites across NI, and in every case, it appeared to be associated with recently planted material (McCracken et al., 2017).

In July 2013, the 'All-Ireland Chalara Control Strategy' was launched (DARDNI, 2013). The strategy of the Irish and Northern Irish Government departments was to eradicate the pathogen and to gather evidence to support an application for Protected Zone status for the pathogen under the EU Plant Health Directive. In 2013, in both jurisdictions ash plants coming from known infected areas were barred from being imported, and any infected tree was destroyed under statutory notice.

Ash is normally planted as 1-year-old bare rooted whips grown from seed. Local production of ash whips in NI is limited so that the majority of planting material was imported either from Great Britain or from countries in Northern Europe. When planted in late winter or early spring no leaves or rachises are present on the plants. It was therefore assumed that if the pathogen had been present it resided in infected wood. Gross et al. (2012) in a hypothetical life cycle of the pathogen had proposed that leaf infection was initiated only by ascospores released from apothecia that had developed on rachises on the forest floor. If this were correct, then, in the absence of leaves, it was not clear how the pathogen could move from infected wood to spore production.

The aim of the study was to investigate the appearance and development of ash dieback at a recently planted commercial ash plantation near Randalstown, Co. Antrim and to use NI-wide data to provide context.

2 | MATERIALS AND METHODS

2.1 | Case study site

In August 2015, ash dieback was confirmed on a commercial planting near Randalstown in Co. Antrim, NI. The mixed woodland site of around 300 m^2, containing approximately equal numbers of European ash (*F. excelsior*), European beech (*Fagus sylvatica*) and common hazel (*Corylus avellana*), had been planted in 2008. All of the saplings had been imported from Great Britain. The soil was heavy clay with restricted drainage. The average annual rainfall at the site was 941 mm leading to some waterlogging and surface water during heavy rain (Meteorological Office, 2015). When first surveyed in late August 2015 most ash trees on the site had some evidence of *H. fraxineus* infection. A Statutory Destruction Order issued under the Plant Health Order (NI Gov, 2006) was placed on the site by DARD and so all subsequent observations and measurements had to be carried out in September and October before the trees were removed and destroyed.

The case study site comprised a mixed plantation in which there were approximately 1000 ash trees of which 234 were selected for more detailed observation. Trees numbered 1–180 were assessed for disease by estimation of the following: leaf spotting; shoot death; stem lesions; basal lesions and general tree health. Further details of the criteria used are provided in Table 1. In addition to trees 1–180 with basal lesions, selected trees, 181–234 similarly infected, were included in the basal lesion study.

Eleven trees were chosen for more intensive study. These trees were selected to represent the full range of symptoms observed in

TABLE 1 Criteria used to assess the level of ash dieback of a single ash tree

Symptom	Measurement/assessment
Leaf symptoms	Viewing the tree canopy (standing on the ground) from all angles, the percentage of leaves showing symptoms (spotting, wilting or discoloration) was estimated using a percentage scale (0, 1, 5, 10, 15, 20, 25, continuing in 5% increments to 100%).
Shoot symptoms	As for leaf symptoms, the percentage of shoots showing symptoms (lack of leaves and/or discolouration) was estimated, using a percentage scale (0, 1, 5, 10, 15, 20, 25, continuing in 5% increments to 100%)
Stem symptoms	Stem symptoms were counted (lesions on the trunk of the tree). The numbers of lesions or discolouration were noted
Basal lesions	The presence or absence of basal lesions was noted
Tree alive or dead	Whether each tree was alive or dead was recorded

the plantation. On first examination of the plantation, ash trees had been sequentially assigned an identification number from which their position within the plantation could be mapped and recorded. The trees were dug up, harvesting as much of the root system as possible, and brought back to the laboratory. Each whole tree was carefully examined noting the position and extent of any lesions, including basal lesions.

In addition, 16 randomly selected trees with large basal lesions were harvested by cutting them at ground level. In the laboratory, the stem was cut through horizontally at the mid-point of the lesion and again 25 mm above to give a transverse slice. This was repeated until no staining was seen within the tissue. The deformation and staining within the wood were compared to similar sections of stems from healthy trees of the same age taken from the site. The age of the tree was determined using the growth rings and the date of infection estimated by examining the position of the staining in relation to the tree rings.

Root systems of trees with basal lesions were brought to the laboratory where they were washed and the presence of any pseudosclerotia noted. Roots, including those with and without pseudosclerotia, were placed in sealed clear plastic containers lined with damp tissue and incubated in the light, at room temperature. The percentage area of the roots covered with pseudosclerotia was estimated visually. Apothecial formation was assessed 7–10 days later. Where apothecia were present, three were selected at random from each of the roots and tested by qPCR (EPPO, 2013; Ioos & Fourrier, 2011) to confirm that they were *H. fraxineus*.

On trees with basal lesions, horizontal cross sections were cut along the length of the stem starting at the visible edge of the lesion. Where the basal lesion had originated from an infection higher up the stem then clear staining was observed indicating that the pathogen had most probably moved downwards. If there was no observable staining in sections cut from above the basal lesion, it was assumed that the point of infection had been at or near ground level.

It was of particular interest to determine as far as possible whether an individual tree had been infected with *H. fraxineus* at the time of planting. The age of the tree could be determined by examining tree rings and confirmed by consulting the growers' planting records. The location of lesions in relationship to tree rings allowed an accurate estimation of when the initial infection had occurred. Figure 1 illustrates the contrast between a healthy (no obvious lesions) and trees infected at the time of planting. Lesions developing from infected planting material were clearly distinguishable from lesions which had developed through leaf or basal infections. The size and growth status of the individual trees were also considered: trees assessed as being infected at an early stage of growth were distinctively small and often dead. The observations made on individual trees at this site also tied in with the observations made at multiple sites over several years where young trees had died a few years after planting and there was no associated evidence of pathogen sporulation.

Using all of these data and observations, it was possible to propose a probable year of infection for individual trees and suggest a timeline for disease development within the plantation.

FIGURE 1 Comparison of cross sections of the stem of a healthy tree (on left) with those of trees which were infected at the time of planting. All stem section are taken from trees of the same age.

2.2 | DARD survey programme

In the 2000s, Plant Health Inspectors from the Department of Agriculture, Environment and Rural Affairs were looking for symptoms on ash plants during their routine inspections of nurseries and planting sites in NI. When the disease was detected in southern England in early 2012 inspections became more focused and young ash plantings in NI were identified and systematically inspected. Furthermore, following the confirmation of the presence of ash dieback in Co. Leitrim, ROI, which is <20 km from the NI/ROI border, more numerous and structured survey inspections were conducted.

At each site inspected, random samples were taken from several ash trees with symptoms of dieback (leaf spotting, wilting, discoloured shoots, etc.) and submitted to the Plant Pathology Diagnostic Laboratories of the Agri-Food & Biosciences Institute (AFBI) in Belfast, NI. Samples were tested for the presence of *H. fraxineus* by qPCR (EPPO, 2013; Ioos & Fourrier, 2011). Following confirmation of the presence of *H. fraxineus* at a site, the landowner was served with a statutory notice requiring all ash trees in the planting to be removed and destroyed. Furthermore, DARD Plant Health Inspectors carried out an intensive inspection of hedgerows, gardens, plantings within a 500 m radius of the infected site looking for ash dieback symptoms. They also carried out a trace back and trace forward exercise to identify the source of the planting material and to identify other sites which had been planted from the same source. Routine inspections of nurseries, recent plantings and in the wider environment were increased. Follow-up visits were made in subsequent years, especially to recently planted sites and to areas close to previous positive sites. Hedgerows and other plantings within a 500 m radius were again checked for ash dieback symptoms. Intensive surveys were conducted in Co. Fermanagh in the west of NI as this is the county bordering Co. Leitrim in ROI. At risk areas of special scientific interest (ASSIs) were also especially targeted.

From 2016, the presence of ash dieback in the wider environment was mapped using the Irish 10 km square grid system (Ordnance Survey of Ireland, 1996), where each square was surveyed and the presence or absence of infected trees was recorded.

3 | RESULTS

3.1 | Randalstown case study site

Of the trees 1–180 examined in the Randalstown site, only three (2%) did not have some symptoms of ash dieback (Table 2). Most commonly observed symptoms were leaf spotting and shoot dieback consistent with ascospore infections having occurred during the current growing season (i.e. 2015). Although lesions and spotting of ash leaves can be due to multiple causes, in a site known to have high levels of ash dieback, confirmed by frequent PCR testing, it was considered that most of the leaf spotting observed was due to *H. fraxineus*. Twenty one percent of trees had stem lesions, 4% had basal lesions and 4% of trees were dead (Table 2). Of the 180 trees, it was estimated that around 13% had been infected when planted.

3.1.1 | Trees with basal lesions

Eighteen of the 234 trees examined had large basal lesions. In some instances, there was clear stem staining between basal lesions and lesions further up the stem as described in Section 2. Such basal lesions were attributed to growth of the pathogen through the plant. However, following dissection of the lesions and surrounding tissue, in most cases, basal lesions were the only lesions observed on the stem. Hence, initial infection had probably not been initiated by leaf and stem infections but had occurred at or near ground level. Apothecia were sometimes observed developing on basal lesions in the field although this was infrequent. While we did not test specifically for *Armillaria*, on no occasion in the field or on plants brought back to the laboratory did we observe any evidence (e.g. rhizomorphs) of this pathogen. We were confident that the death of the trees we examined was, certainly in the first instance, due to a *H. fraxineus* infection.

When roots with pseudosclerotia were incubated in damp conditions in the laboratory, numerous apothecia developed along their lengths within 7–10 days (Figure 2). Apothecia developed from roots of six out of 18 trees with pseudosclerotia (Table 3; Figure 2) and all that were tested (three per root) were confirmed as *H. fraxineus* by qPCR.

3.1.2 | Intensive study of 11 selected diseased trees

The disease symptoms observed on each of the 11 intensively studied trees ranged from limited leaf spotting to severe stem lesions and tree death (Table 4).

When the trees were examined in the autumn of 2015, one (Tree 163) had only leaf symptoms, resulting from infections occurring during summer 2015. The inoculum for these infections had been produced by apothecia which had probably been formed during 2014 or spring 2015. There were no stem lesions, basal lesions or any significant shoot dieback. This tree had most likely been free of infection at the time of planting and had grown uninfected for several years before air-borne ascospores had landed and germinated on healthy leaves.

On Tree 169 a single large stem lesion was observed at a height of 45 cm above ground level. This lesion was associated with a side shoot which was estimated from tree ring counts to have been infected 4 years previously during summer 2011. The infected side shoot was dead and covered with a dry papery epidermis. On several trees which had been severely infected in previous seasons the authors observed that many of the smaller dead branches were covered with a similar pale papery epidermis. When this was peeled away pseudosclerotia were observed (Figure 3) and often apothecia had started to develop beneath this epidermis.

TABLE 2 Percentage of trees with different types of symptoms of ash dieback in September 2015 (many trees had multiple symptoms) based on 180 trees.

Symptom type	Trees affected (%)
Healthy	2
Leaf spotting or wilting	72
Side shoot dieback	67
Main stem lesion	21
Basal lesion	4
Dead	4
Planting material infected	13

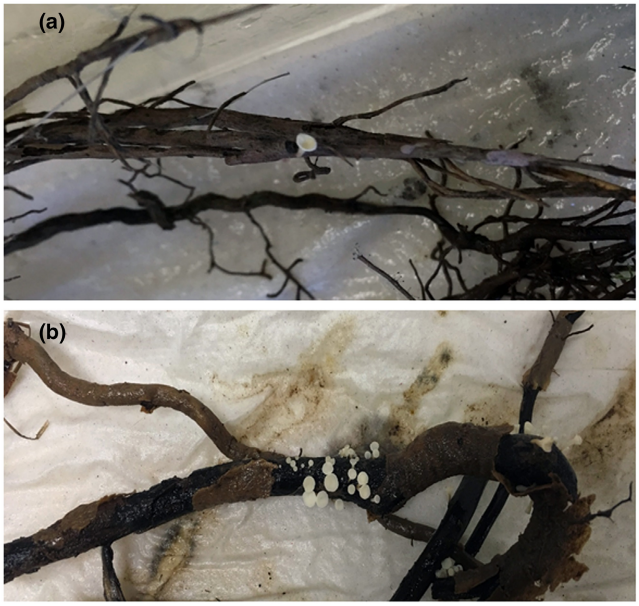

FIGURE 2 *Hymenoscyphus fraxineus* apothecia on the roots of two infected ash trees (a, b). Note that roots are covered with pseudosclerotia.

Tree 61 was one of the most severely affected trees. Three large lesions had formed towards the base. The lesions had girdled the main stem resulting in death of the uppermost part of the tree. Apothecia were also observed on basal lesions, both in the field and after a period of incubation in the laboratory. The site where trees were growing was wet and the base of trees was surrounded by grass. There was extensive epicormic growth from the base of the tree. When the sections of the lesions were examined there was clear evidence that the symptoms had developed soon after the tree had been planted. The tree was deemed to have been infected during propagation so that the whip was already infected at the time of planting.

Tree 90 was the only 1 of the 11 extensively studied trees which had a basal lesion, but the lesion had not girdled the stem. There was no evidence of epicormic growth from the base of the tree and there was extensive leaf flush. However, around 75% of leaves showed some spotting or wilting. Approximately 75% of shoots had necrotic lesions. Leaf infections and probably most shoot infections had occurred during summer 2015. Sections of the basal lesion indicated that the tree was not infected before planting but that this infection had occurred 2 years previously during 2013.

TABLE 3 *Hymenoscyphus fraxineus* apothecia production on the roots and stems of dead ash trees that had basal lesions following incubation in the laboratory.

Tree number	Pseudosclerotia (% cover)	Average no. of apothecia (number/cm^2)
3	100	1
25	50	0
26	25	0
33	50	0
63	<10	0
91	25	0
93	75	0
95	50	2
187	20	6
188	100	0
189	50	3
196	<10	0
198	25	0
208	100	16
218	80	0
230	<10	0
232	80	1
234	100	0

3.2 | DARD surveillance

In 2012/2013, DARD plant health inspectors carried out 2923 site inspections for ash dieback (Table 5). Ash dieback was found at 91 sites, including three nurseries. At all of the sites where the disease was found the trees had been planted, as far as records could show, during or after 2006. During 2012/2013, on no occasion was ash dieback diagnosed in the immediate (500 m radius) environment of positive findings. Visual buffer surveys continued at each site in subsequent years and by 2018 and 2019 infections within hedgerows were common and severe (DAERA Plant Health Inspectorate, Personal Communication).

From 2012 to the end of 2015, the majority of cases (73%) had been found in recently planted woodland plantations with the remainder being detected in urban/amenity settings, private gardens, roadsides, recently planted hedges and, in only three cases, in plants in nurseries (Table 5).

Following the discovery in 2016 of ash dieback in the wider environment i.e. in hedgerows, trees planted before 2006 and in areas of regeneration from naturally dispersed seed, DAERA adopted a

Tree no.	Height (m)	Leaf symptoms (%)	Shoot symptoms (%)	No. stem lesions	Basal lesion	Date of 1st infection
163	6.5	25	25	0	0	2015
56	5	<10	25	0	0	2013
142	4	<10	10	2	0	2013
52	2.5	<10	10	2	0	2013
90	3.5	75	75	1	1	2013
1308	3.5	<10	10	3	0	2012
169	2.5	<10	50	1	0	2011
129	4	25	50	2	0	2012
111	3.5	25	75	1	0	2008
168	2.5	<10	10	2	0	2008
61	3.3	75	75	3	0	2008

Note: Leaf symptoms are an estimate of the percentage of leaves with symptoms. The date of first infection was estimated from the location of symptoms within each tree and the extent of their development (e.g. if only leaf symptoms were observed then first infection had occurred during the current growing season).

TABLE 4 Height and ash dieback symptoms of each of 11 intensively studied trees.

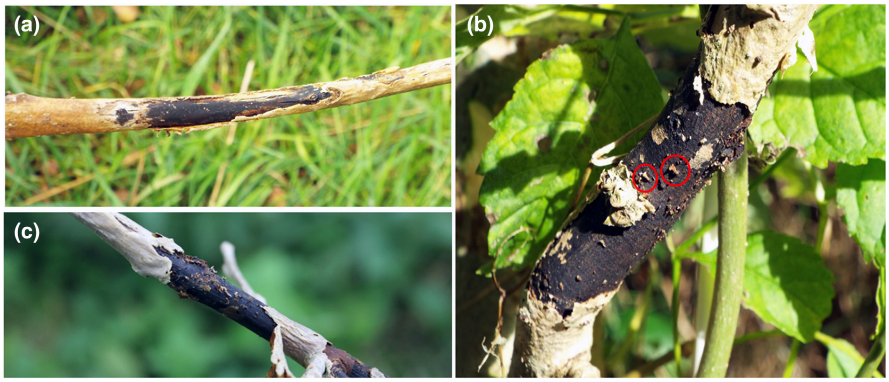

FIGURE 3 Stems, still attached to three different trees (a–c) trees showing the development of pseudosclerotia beneath papery epidermis, which when removed revealed fully formed apothecia (see circled areas).

TABLE 5 Confirmed findings (as of December 2016) of ash dieback (caused by *Hymenoscyphus fraxineus*) in Northern Ireland (DAERA, 2021).

	Inspections	*H. fraxineus* confirmed at planting site (premises)	*H. fraxineus* confirmed at nursery/retail/ trade site	Total
2012	1028	76	3	79
2013	1895	12	0	12
2014	1306	2	0	2
2015	1896	18	0	18
2016	2680	68	0	68
Total	8805	176	3	179

different approach to surveying for the disease and to presenting the data. As of 31October 2018, the disease had been found in all but five of the 10 km grid squares in NI, with the majority of incidences being in 2018 (DAERA, 2021). Observations by the authors of this paper have indicated that by the autumn of 2020 ash dieback was present on all ages of ash trees throughout NI.

4 | DISCUSSION

On the basis of the findings of this study, a probable timeline of the introduction and development of ash dieback in NI was developed (Figure 4). The introduction of the ash dieback pathogen *H. fraxineus* into NI was on infected planting material. Despite very intensive and widespread surveys by plant health inspectors in both Northern Ireland and the Republic of Ireland symptoms of ash dieback had not been detected on the island of Ireland until late 2012. As we have shown, these lesions most probably developed on plants which had been infected at the time of planting. At none of these early detected sites were apothecia found on rachises indicating that the pathogen had not yet entered its phase of production of aerial spores. We are therefore confident that inoculum production had not occurred prior to 2012 as shown in Figure 4. At the Randalstown site, trees displayed a wide range of symptoms from leaf spotting to large stem lesions. During the period 2012–2015 spore traps had been placed close to two infected NI sites and at a further two locations in the east of NI where deposition of air-borne spores would have occurred if they had originated in Great Britain, as predicted by mathematical models developed at the University of Cambridge

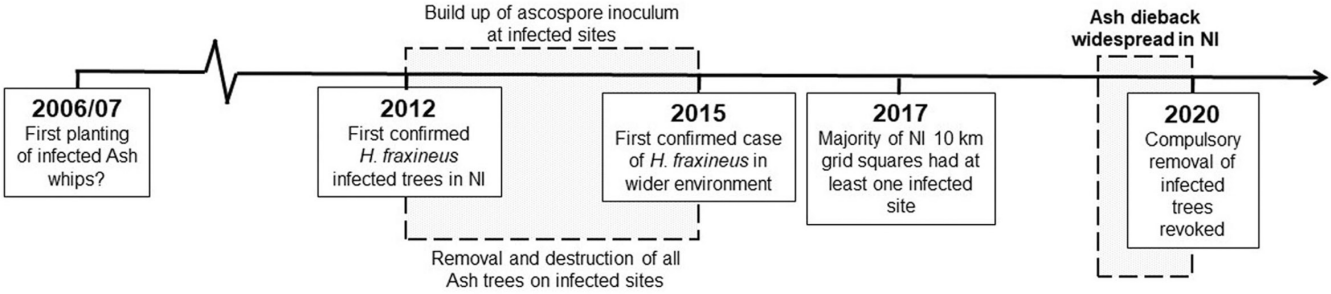

FIGURE 4 Probable timeline of the introduction and development of ash dieback in Northern Ireland.

(McCracken et al., 2017). Spore traps were examined weekly over a 4-month (August–November) period in each year. No ascospores were detected during microscopic examination of the sampler slides (A. R. McCracken, unpublished data).

What is less clear is when the first infected plants were brought to Northern Ireland. In the majority of cases in 2012, the infected plantations were ≤6 years old. This would suggest that the first infections were introduced on planting material imported in or after 2006. The plantation described in this paper was planted in winter 2008/2009. Wylder et al. (2018), using evidence from tree rings, supported by PCR detection of *H. fraxineus*, concluded that the pathogen was present at some sites in England as early as 2004/2005.

Despite intensive surveying of 500 m radius buffer zones around every site in NI where ash dieback was confirmed, both at the time of detection and in subsequent years, no symptoms were detected in these buffer zones until summer/autumn 2015. This would suggest that active inoculum had been produced in summer 2014 or 2015 in sufficient quantity to initiate new infections. Timmermann et al. (2011) concluded that once the ash dieback pathogen is in the wider environment, it becomes almost impossible to control its spread.

Roots may play a role in the infection of young trees. Schumacher (2011) presented evidence that the fungus was able to spread very effectively in wood as a parasite but did not originate from the root system. Kowalski and Łukomska (2012) reported isolating *H. fraxineus* from infected roots of living plants, but we believe that the present study is the first report of the formation of apothecia on roots. Fones et al. (2016) considered that the asexual spores (conidia) of *H. fraxineus* have a role in the infection of ash trees; they showed that *H. fraxineus* can survive and grow in soil inoculated with conidia and that the conidia are capable of germinating, infecting and colonizing ash seedlings via roots. Thus infection of the ash tree roots observed in our study may have been initiated by *H. fraxineus* mycelium or germinating spores from leaf litter on the soil surface or even within the soil.

Several studies have indicated that wet, waterlogged sites predispose ash trees to the development of basal (also called collar) rots (Enderle et al., 2013; Husson et al., 2012; Marçais et al., 2016). Marçais et al. (2016) found a correlation between ash dieback canker severity and high soil moisture: sites with high soil moisture or in more humid topographical positions were associated with more severe basal lesions. Husson et al. (2012) reported that collar lesions were significantly larger in young ash stands in plots with high moisture levels and commented that these conditions may favour survival of the pathogen on ash rachises and production of apothecia. These authors postulated that collar infections probably played a major role in the sudden decline and mortality of mature ashes in eastern France as they found that the severity of collar lesions and crown decline were significantly correlated in the severely affected stands. Husson et al. (2012) further discussed the role of lenticels as efficient infection pathways through which the spores of pathogens can attack hosts and suggested that basal lesions and root infections were induced by ascospores that were able to infect the stem base through lenticels. Nemesio-Gorriz et al. (2019) reported that lenticels can act as entry points for fungal pathogens, including *H. fraxineus*, through the bark of ash shoots. The authors of the present paper frequently observed that in the damp microclimate around the base of trees in the Randalstown site lenticels were prominent.

When ash dieback was diagnosed on a site in NI in the period 2012–2016, growers were required to remove all ash trees immediately. With the exception of very young trees, the removal was done by cutting off the plants at the ground level and taking away all above-ground parts, leaving the roots to rot naturally, assuming that the pathogen did not survive or sporulate within the roots. The observations from this current case study provide evidence that not only did roots become infected, as reported by Kowalski and Łukomska (2012), but that apothecia form on them. Infected roots that remained in the ground after removal of ash trees and the distribution of logs or wood chips from infected trees both pose a theoretical risk of spreading *H. fraxineus*, but these are unlikely to be significant in practice. It was recognized that removal of rachises at the time of plantation clearance may have given extra protection. Physical removal was considered but was impractical. Investigations by one of the authors of this paper (Baxter, 2022) and by Tiley and O'Hanlon (2022) on the use of urea to accelerate degradation of rachises before apothecia could form were not successful.

Formation of apothecia on small stems still attached to growing trees was considered a potential source of inoculum. Apothecia appeared to start developing from areas of pseudosclerotia beneath papery epidermis of these stems. Being some distance above ground, dispersal of ascospores to neighbouring, uninfected trees

would readily occur. During this study, apothecia were found forming below the epidermis of small 1-year-old twigs still attached to the tree. The epidermis became papery and was easily removed by wind, heavy rain or other mechanical means potentially enabling the apothecia to release their ascospores. The rainfall at this site was relatively high (>900 mm/year), which is not atypical for rainfall levels in the east of N. Ireland, which could have encouraged apothecial development which may not occur in drier conditions. Development of apothecia on infected roots was also observed (although only after incubation in the laboratory). Apothecial development on either or both of these sites within these imported infected trees could have provided inoculum enabling *H. fraxineus* to spread into the wider environment. Further investigation of potential locations of apothecial production on infected ash trees is warranted.

This study shows that the twin aims of eradication and establishing protected zone status were not achieved and that despite initial efforts, the 'Eradication and Containment' policy for ash dieback was unable to meet its objectives. This was apparently due to a combination of factors, including the epidemiology of the disease (fungal pathogens spread through airborne spores are notoriously difficult to contain once established), the timing of the actions taken, which were demonstrably later than ideal and an incomplete scientific understanding of the pathology of the fungus in question. There are many elements involved in preventing the establishment of a pest or pathogen in a region where it is not endemic. First there is the need for an early warning system. The movement of ash dieback westwards and northwards had been well documented since it was first diagnosed in Poland in 1996 (Przybyl, 2002). While initially there was some confusion over the organism responsible, it was soon clear that its spread was both rapid and devastating. The UK and Ireland were therefore aware of the risk posed by this pathogen and, as a result, initiated surveys of ash planting sites. This resulted in the first report of ash dieback in Kent in early 2012. However, further research has suggested that the pathogen had been present in England since as early as 2004 (Wylder et al., 2018). Ash dieback was first found in ROI in October 2012 and in NI in November 2012 and further infected sites were soon identified. It could be argued that if the UK and Ireland had banned the import of ash plants from areas where the disease was present prior to 2012, perhaps in 2007/2008 when the spread of the disease across Europe was recognized, then the ingress of the pathogen might have been halted or at least delayed, allowing more time for the identification of potentially resistant trees. In response to a number of plant disease outbreaks, the UK has since developed a Pest Risk Register (DEFRA, 2014), which currently lists over 1200 pests. This should, in theory, identify the organisms which present the highest risk to UK agriculture, horticulture, forestry and amenity.

The attempt to eradicate *H. fraxineus* from the island of Ireland, despite warnings of probable failure from colleagues (e.g. T. Kiristis, Personal Communication) in the rest of Europe in countries where ash dieback was already established, was based on the then understanding of the pathogen's lifecycle. The lifecycle described by Gross et al. (2012) indicated that the primary, perhaps only, mechanism of spread, apart from movement of infected plants, was by the dissemination of sexual ascospores, developing from apothecia formed on leaf rachises on the forest floor. Asexual conidia were considered to have no active role. All of the survey data and observations of the case study site presented here indicated that the pathogen had been present in 1–2-year-old ash whips at the time of planting in 2008 and subsequently grew and produced stem lesions, on occasions killing the plant within 5 or 6 years. The present study has shown how it is possible for apothecia to develop on such plants and initiate spread of ash dieback.

ACKNOWLEDGEMENTS

One of the authors (EB) thanks DARD/DAERA for funding her studies under their 2012 Postgraduate Studentship competition. The authors acknowledge the work done by a large team of plant health inspectors. Thanks are also due to the AFBI laboratory diagnostic staff under the supervision of Dr Colin Fleming and members of the Forest Service of Northern Ireland including the Director, Mr Malcolm Beattie. Thank you to Mrs Diane Stevenson, DAERA for advice, particularly about plant health legislation. The authors appreciate the willingness of the landowners to allow staff and students access the site to carry out observations and studies. The authors also thank Dr Anna Tiley and Dr Richard O'Hanlon for their helpful comments on the manuscript during its preparation.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Louise R. Cooke https://orcid.org/0000-0002-2761-3689
Irene R. Grant https://orcid.org/0000-0001-5860-3769

REFERENCES

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

Baxter, E. (2022). *The epidemiology of the ash dieback pathogen*, Hymenoscyphus fraxineus, *in Northern Ireland* (241 pp.). Thesis submitted for the Degree of Doctor of Philosophy in the School of Biological Sciences Queen's University Belfast.

Chandelier, A., Helson, M., Dvorak, M., & Gischer, F. (2014). Detection and quantification of airborne inoculum of *Hymenoscyphus pseudoalbidus* using real-time PCR assays. *Plant Pathology*, *63*, 1296–1305.

Cleary, M., Nguyen, D., Stener, L. G., Stenlid, J., & Skovsgaard, J. P. O. (2017). Ash and ash dieback in Sweden: A review of disease history, current status, pathogen and host dynamics, host tolerance and management options in forests and landscapes. In R. Vasaitis & R. Enderle (Eds.), *Dieback of European ash (Fraxineus spp.) – Consequences and guidelines for sustainable management* (pp. 195–208). The report on the European Cooperation in Science and Technology (COST) Action FP1 103 FRAXBACK. Swedish University of Agricultural Sciences.

DAERA. (2021). *Ash dieback: Current situation in Northern Ireland*. http://www.daera-ni.gov.uk/articles/ash-dieback#toc-0

DARDNI. (2013). *All-Ireland Chalara control strategy*. https://www.dardni.gov.uk/publications/all-ireland-chalara-control-strategy

DEFRA. (2014). *UK Plant Health Risk Register.* https://secure.fera.defra.gov.uk/phiw/riskRegister/downloadEntireRiskRegister.cfm

Drenkhan, R., & Hanso, M. (2010). New host species for *Chalara fraxinea*. *New Disease Reports*, *22*, 16.

Enderle, R., Peters, F., Nakou, A., & Metzler, B. (2013). Temporal development of ash symptoms and spatial distribution of collar rots in a provenance trial of *Fraxinus excelsior*. *Journal of Forest Research*, *133*, 865–876.

Enderle, R., Stenlid, J., & Vasaitis, R. (2019). An overview of ash (*Fraxinus* spp.) and the ash dieback disease in Europe. *CABI Reviews*, *14*, 1–12.

EPPO. (2013). PM 7/117 (1) *Hymenoscyphus pseudoalbidus*. *EPPO Bulletin*, *43*, 449–461.

EPPO. (2022). *Hymenoscyphus fraxineus (CHAAFR) distribution*. EPPO Global Database. https://gd.eppo.int/taxon/CHAAFR/distribution

Fones, N. H., Mardon, C., & Gurr, S. (2016). A role for the asexual spores in infection of *Fraxineus excelsior* by the ash-dieback fungus *Hymenoscyphus fraxineus*. *Scientific Reports*, *6*, 1–10.

Grosdidier, R., Loos, R., Cael, O., Scordia, T., & Marcais, B. (2018). Tracking the invasion: Dispersal of *Hymenoscyphus fraxineus* airborne inoculum at different scale. *FEMS Microbiology Ecology*, *94*, 1–11.

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

Heinze, B., Tiefenbacher, H., Litschauer, R., & Kirisits, T. (2017). Ash dieback in Austria – History, current situation and outlook. In R. Vasaitis & R. Enderle (Eds.), *Dieback of European ash (Fraxineus spp.) – Consequences and guidelines for sustainable management* (pp. 33–52). The report on the European Cooperation in Science and Technology (COST) Action FP1 103 FRAXBACK. Swedish University of Agricultural Sciences.

Husson, C., Cael, J. P., Grandjean, L. M., & Marcais, B. (2012). Occurrence of *Hymenoscyphus pseudoalbidus* on infected ash logs. *Plant Pathology*, *61*, 889–895.

Ioos, R., & Fourrier, C. (2011). Validation and accreditation of a duplex real-time PCR test for reliable *in plant* detection of *Chalara fraxinea*. *EPPO Bulletin*, *41*, 21–26.

Kirisits, T., Matlakova, M., Mottinger-Kroups, S., Cech, T. L., & Halmschlager, E. (2009). The current situation of ash dieback caused by *Chalara fraxinea* in Austria. In T. Doğmuş-Lehtijärvi (Ed.), *Proceedings of the conference of IUFRO Working Party 7.02.02, Egirdir, Turkey, 11-16 May 2009*. (pp. 97–119) Serial: A, Special Issue. SDU Faculty of Forestry Journal. ISSN: 1303-7085.

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

Kowalski, T., & Łukomska, A. (2012). The studies of ash dying (*Fraxineus excelsior* L.) in the Włoszczowa Forest Unit stands. *Acta Agrobotanica*, *58*, 429–440.

Marçais, B., Husson, C., Godart, L., & Caël, O. (2016). Influence of site and stand factors on *Hymenoscyphus fraxineus*-induced basal lesions. *Plant Pathology*, *65*, 1452–1461.

McCracken, A. R., Douglas, G. C., Ryan, C., Destefanis, M., & Cooke, L. R. (2017). Ash dieback on the Island of Ireland. In R. Vasaitis & R. Enderle (Eds.), *Dieback of European ash (Fraxineus spp.) – Consequences and guidelines for sustainable management* (pp. 125–139). The report on the European Cooperation in Science and Technology (COST) Action FP1 103 FRAXBACK. Swedish University of Agricultural Sciences.

Meteorological Office. (2015). *Historic station data [online]*. https://www.metoffice.gov.uk/research/climate/maps-and-data/historic-station-data

Nemesio-Gorriz, M., McGuiness, B., Grant, J., Dowd, L., & Douglas, G. C. (2019). Lenticel infection in *Fraxineus excelsior* shoots in the context of ash dieback. *iForest*, *12*, 160–165.

NI Gov. (2006). *Plant Health Order (N) 2006*. https://www.legislation.gov.uk/nisr/2006/82/contents/made

NI Gov. (2018). *The Plant Health (amendment no. 3) Order (Northern Ireland) 2012 amending The Plant Health Order (NI) 2006 making it the Plant Health Order (NI) 2018*.

Ordnance Survey of Ireland (Director). (1996). *The Irish grid. A description of the co-ordinate reference system used in Ireland*. https://osi.ie/wp-content/uploads/2015/04/The-Irish-Grid-A-Description-of-the-Coordinate-Reference-System-Used-in-Ireland.pdf

Pastirčaková, K., Adamčiková, B. M., Pažitný, J., Hoťka, P., Sarvašová, I., & Horáková, K. (2020). Host range of *Hymenoscyphus fraxineus* in Slovak arboreta. *Forests*, *11*, 1–18.

Przybyl, K. (2002). Fungi associated with necrotic apical parts of *Fraxinus excelsior*. *Forest Pathology*, *32*, 387–394.

Rozsypálek, J., Dvořák, R., Longauer, R., Botella, L., Prouza, M., Palovčíková, D., & Jankovský, L. (2017). Ash and ash dieback in the Czech Republic. In R. Vasaitis & R. Enderle (Eds.), *Dieback of European ash (Fraxineus spp.) – Consequences and guidelines for sustainable management* (pp. 70–88). The report on the European Cooperation in Science and Technology (COST) Action FP1 103 FRAXBACK. Swedish University of Agricultural Sciences.

Schumacher, J. (2011). The general situation regarding ash dieback in Germany and investigations concerning the invasion and distribution strategies of *Chalara fraxinea* in woody tissue. *EPPO Bulletin*, *41*, 7–10.

Tiley, A., & O'Hanlon, R. (2022). Living with the impact of ash dieback – Local mitigation practices against *Hymenoscyphus fraxineus* on the Island of Ireland. *Preprint*, *2022*, 2022010351. https://doi.org/10.20944/preprints202201.0351.v1

Timmermann, V., Borja, I., Hietala, A. M., Kirisits, T., & Solhei, H. (2011). Ash dieback: Pathogen spread and diurnal patterns of ascospore dispersal with special emphasis on Norway. *EPPO Bulletin*, *4*, 14–20.

Wylder, B., Biddle, M., King, K., Baden, R., & Webber, J. (2018). Evidence from mortality dating of *Fraxineus excelsior* indicates ash dieback (*Hymenoscyphus fraxineus*) was active in England 2002–2005. *Forestry*, *91*, 434–443.

Yearsley, J. (2016). *Assessing Ireland's risk to airborne spread of ash dieback disease with Lagrangian stochastic models (MASAD)*. CoFoRD. https://assets.gov.ie/134623/2cf01493-2452-4b4f-b0f7-06b64c89daa3.pdf

How to cite this article: Baxter, E., Cooke, L. R., Spaans, F., Grant, I. R., & McCracken, A. R. (2023). The introduction of *Hymenoscyphus fraxineus* to Northern Ireland and the subsequent development of ash dieback. *Forest Pathology*, *53*, e12789. https://doi.org/10.1111/efp.12789