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Living with the impact of ash dieback – local mitigation practices against *Hymenoscyphus fraxineus* on the Island of Ireland

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Running Title:
A review of the current and future strategies to mitigate the impact of ash dieback under Irish conditions.

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Ash, ash dieback, disease management, *Fraxinus excelsior*, fungal plant pathogen, *Hymenoscyphus fraxineus*, mycology, plant pathology, plant pathogen, plant science, tree disease

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1. Abstract

Ash trees have considerable economic, cultural and environmental value on the island of Ireland. However, European ash (*Fraxinus excelsior* L.) is currently under threat from the invasive ascomycete pathogen *Hymenoscyphus fraxineus*. This pathogen is the causal agent of ash dieback disease, which was initially reported in Poland in 1992. *Hymenoscyphus fraxineus* has since spread across Europe and the first recorded case of the disease on the island of Ireland was in 2012 at a forestry plantation in Co. Leitrim. The pathogen is now present in all 26 counties in Ireland and 6 counties in Northern Ireland, and it is considered unfeasible to eradicate. The spread of ash dieback disease is reflected in recent policy changes, which focus on management rather than eradication strategies.

Since the first formal description of *H. fraxineus* in 2006, considerable research efforts have been made by the international scientific community to understand the biology of the pathogen and to develop management strategies against it. This review provides an update of current knowledge of *H. fraxineus* biology and infection. We then explore examples of mitigation techniques that have been trialled in Europe, in order to identify strategies that are feasible for disease management at a local level on the island of Ireland. Finally, we outline five key avenues of research that have the potential to provide breakthroughs in methods to protect valuable *F. excelsior* resources.

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2. Introduction

European ash (*Fraxinus excelsior* L.) is a widespread tree on the island of Ireland. Spaans *et al.* (2018) estimate that almost 57% of hedgerow trees in Northern Ireland are *F. excelsior*. As a forest species, *F. excelsior* is the 2nd most common broadleaf species, with over 25,000 ha (ca. 4% forest estate) planted (NFI 2017), and it is a component of >90% of native woodlands (McCracken *et al.*, 2017). The National Biodiversity Data Centre maps *F. excelsior* as the 2nd most commonly recorded tree species, after *Crataegus monogyna* Jacq. (NBDC 2021).

Fraxinus excelsior also has social, cultural and biodiversity importance on the island of Ireland. From a historic point of view *F. excelsior* was a very significant tree, and variations of the common name for *F. excelsior* are found in more than thirteen town names in Ireland. This indicates that the tree held high importance in the Irish society around the period 1,000 AD (McLoughlin 2016). From a present day societal point of view, the timber of *F. excelsior* is highly prized as the raw material is used for crafting the hurley (or hurl), which is a piece of equipment used in playing the Irish sport of hurling. There is an annual requirement for 360,000 hurleys in Ireland, which needs around 2000m^3 of ash timber to manufacture (McCracken *et al.*, 2017). The tree species also provides a habitat for biodiversity, with data from Britain indicating that 953 species are associated with *F. excelsior*. This figure includes 44 obligate species that could not survive without the tree species (Mitchell *et al.*, 2014).

Ash dieback is a serious disease of Fraxinus species, which leads to high levels of tree mortality. The symptoms of ash dieback are summarised in Figure 1. This disease is caused by the fungal pathogen *Hymenoscyphus fraxineus* (Kowalski, 2006; Baral *et al.*, 2014). Kowalski (2006) first formally described the species causing the disease as *Chalara fraxinea*. Later work

by Kowalski & Holdenrieder (2009) discovered that *Chalara fraxinea* was only the anamorph (asexual form) of the species, and the teleomorph (sexual stage) morphologically and molecularly resembled the species *Hymenoscyphus albidus*. In-depth work by Queloz *et al.* (2011) showed that there was some cryptic speciation in the taxon *H. albidus*, and therefore the name of the ash dieback pathogen was changed to *Hymenoscyphus pseudoalbidus*. Finally, Baral et al. (2014) corrected the name to *Hymenoscyphus fraxineus*, according to the international rules of biological nomenclature.

The pathogen was first detected in Ireland and in Northern Ireland in 2012 (DAERA 2021). This was after findings of the pathogen in Britain in 2012 (Forest Research 2022) and several other European countries since the first discovery in Poland in the 1990s (Kowalski, 2006). There is growing evidence that the pathogen may have been moving on infected asymptomatic *F. excelsior* plants, undetected, in many European countries before its formal detection by authorities (e.g. Grosdidier *et al.*, 2018a; Orton *et al.*, 2018; Wylder *et al.*, 2018). While the introduction of the pathogen to the island of Ireland almost certainly took place via infected *F. excelsior* plants for planting, modelling work has shown that it is likely that ash dieback disease would have eventually entered the island of Ireland by natural (airborne spores) means from Britain (Yearsley 2016). The mortality rate of infected *F. excelsior* is high, with analysis of a number of stands indicating between 70 and 85% mortality of trees exposed to the pathogen (Coker *et al.*, 2019).

Ash dieback is now present in every country in Ireland (DAFM 2021) and Northern Ireland (DAERA 2021). Short and Hawe (2018) calculate that the cumulative investment in *F. excelsior* forests in Ireland through government grants is more than €120 million. Between the period of 2012 and 2020, around €7 million had been paid in grants to

landowners to remove diseased *F. excelsior* and replant with another species (DAFM 2020). Data from Britain has estimated that ash dieback disease will cost almost £15 billion to the British taxpayer. Shortly after the detection of the pathogen on the island of Ireland, the governments of Ireland and Northern Ireland developed the All Ireland Chalara Control Strategy (Anon, 2013).This strategy involved an eradication campaign supported by grants, research, consultation and legislative actions to stop further introductions of the pathogen on high risk commodities. In 2018, both governments changed the focus of the response from eradication to management of the disease (DAERA 2018; DAFM 2020). The reason for this was that the disease was now widespread, and eradication of the pathogen was considered very unlikely.

Ash dieback has been present in much of Europe before it finally arrived in Ireland and Northern Ireland. Between the formal description of the pathogen in 2006 and 2020, there has been a large research effort by European and global research teams to fully understand the pathogen and disease (Figure 2). Given the current focus on managing the disease in Europe, this paper will review studies on the disease and provide options for the local management of disease impact. This work will review current mitigation techniques to control *H. fraxineus* and identify some of the knowledge gaps in the scientific understanding of *H. fraxineus* management.

3. The biology of *H. fraxineus*

3.1 Lifecycle

The lifecycle of *H. fraxineus* can be divided into six stages: sexual sporulation, asexual sporulation, colonisation, latent phase, necrotrophic phase and overwintering (Summarised in Figure 3).

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a. Sexual sporulation

During the summer months (typically between June to September), *H. fraxineus* produces sexual fruiting bodies (apothecia) on senesced ash leaf litter from the previous years (Timmermann *et al.*, 2011; Hietala *et al.*, 2013; Dvorak *et al.*, 2016; Grosdidier *et al.*, 2018a; Mansfield *et al.* 2018). These apothecia form from the blackened pseudosclerotial plates that are produced by the fungus and which cover the surface of ash leaf petioles (Kowalski and Holdenrieder, 2009a; Kowalski *et al.* 2013).

Morphologically, the apothecia are composed of a flat disk approximately 1.5 - 3 mm in diameter which is borne on a narrow stipe that is approximately 0.4 - 2 mm long and 0.2 - 0.5 mm wide (Kowalski and Holdenrieder, 2009a; Kowalski *et al.* 2013). The apothecia release sexual spores (ascospores) which are approximately 13 - 17 x 3.5 - 5 µm in size and dispersed by the wind (Gross *et al.* 2014). Under laboratory conditions, ascospores are rarely ejected to a height above 0.5 cm (Mansfield *et al.* 2018). However, the ascospores are an efficient means of long-distance dispersal under natural conditions and they are estimated to spread an average distance of 1.4 - 2.6 km away from the source of inoculum (Grosdidier *et al.*, 2018a). Release of ascospores by *H. fraxineus* has a diurnal rhythm, and the highest deposition rates occur in the early morning (Timmermann *et al.* 2011; Hietala *et al.* 2013; Mansfield *et al.* 2018). Findings by Dvorak *et al.* (2016) and Burns *et al.* (2021) have both identified meteorological variables, such as leaf wetness, influence ascospore production. The timing of ascospore release by *H. fraxineus* may therefore coincide with morning dew formation, which could protect the spores from desiccation and promote germination following contact with the host surface (Hietala *et al.*, 2013; Dvorak, *et al.*, 2016; Mansfield *et al.* 2018).

b. Asexual sporulation

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Asexual sporulation occurs on the leaf surface and has also been observed on dead seedlings and infected wood (Husson *et al.*, 2012). The asexual spores (conidia) emerge as droplets or chains on terminal hyphae or phialides (Kowalski, 2006, Kowalski and Holdenrieder, 2009, Husson *et al.*, 2012, Cleary *et al.* 2013; Fones *et al.* 2016). Kowalski *et al.* (2006) document that each single celled conidia measures approximately 3.2 - 4.0 x 2.0 - 2.5 µm in size. The role of conidia in the lifecycle of *H. fraxineus* is debated, and it has been hypothesised that these spores act as the spermatia (male gametes) for sexual reproduction (Gross *et al.* 2012). However, recent evidence by Fones *et al.* (2016) demonstrated that conidia are capable of causing infection on ash seedlings. Therefore, asexual sporulation may serve to (1) facilitate mating followed by sexual sporulation and (2) act as a secondary source of inoculum.

c. Colonisation

The wind-blown ascospores are the primary source of infection of ash by *H. fraxineus*. These ascospores infect ash leaves (reviewed in Gross *et al.*, 2014) with direct infection of the root collar and infection of aerial roots possibly occurring via lenticels in bark (Husson *et al.*, 2012; Nemesio-Gorriz *et al.*, 2019). Scanning electron microscopy by Cleary *et al.* (2013) revealed that the ascospores attach to the host surface with a mucilage matrix, and they then germinate to produce a single germ tube. The germ tube swells into an adhesive disk that adheres firmly to the leaf surface, enabling penetration of the epidermal cells (Cleary *et al.*, 2013). Mansfield *et al.* (2018) recorded that 80% of the ascospores that germinated did not produce a germ tube, and instead differentiated directly into appressoria. The authors postulated that the lack of requirement of a germ tube for infection could be advantageous to the pathogen, as it reduces exposure of non-melanised hyphae to the environment (Mansfield *et al.*, 2018).

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d. Latent phase

Research using a combination of microscopy and cytology coupled with gene expression analyses and high-throughput sequencing has helped to clarify the early stages of infection by *H. fraxineus*, prior to the first emergence of visible symptoms (Cross *et al.*, 2017; Mansfield *et al.*, 2018; Mansfield *et al.*, 2019). The results from these studies show that *H. fraxineus* has a long asymptomatic phase in which the pathogen accumulates in the leaf tissue as a quiescent endophyte. During this time, the pathogen grows within the host tissue without inducing major physiological changes or visible symptoms (Dal Maso *et al.*, 2012; Cross *et al.*, 2017; Mansfield *et al.*, 2018; Mansfield *et al.*, 2019). Cytological analysis by Mansfield *et al.* (2018) revealed that *H. fraxineus* forms bulbous hyphae within the first penetrated cell and the pathogen then spreads to surrounding tissue. During this stage of infection, the pathogen directly penetrates the epidermal cell wall and produces intracellular hyphae which colonise neighbouring epidermal and subepidermal cells (Mansfield *et al.*, 2018). Further research by Mansfield *et al.* (2019) noted that at approximately 6 days post-inoculation, penetrated plant epidermal cells appeared viable and contained intact plasma membranes and organelles. In addition, the membrane of these plant cells was closely associated with the *H. fraxineus* hyphae. The authors therefore suggested that some of the features of infection by *H. fraxineus* are comparable to those of other hemibiotrophic pathogenic fungi such as *Colleototrichum* species, *Magnaporthe oryze* and *Zymoseptoria tritici* (Mansfield *et al.*, 2019).

In their cytological analysis of infection by *H. fraxineus*, Mansfield *et al.* (2019) also reported increased browning of vacuolar contents in the plasmolysed plant cells ahead of invasion by the pathogen. Therefore, *H. fraxineus* may release virulence factors to aid infection, and these may diffuse ahead of the leading edge of colonisation (Mansfield *et al.*, 2019).

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e. Necrotrophic phase

Following colonisation, there is a breakdown of the biotrophic interaction and *H. fraxineus* switches to a pathogenic/necrotrophic growth phase (Cross *et al.*, 2017; Mansfield *et al.* 2019). Although it is not clear what leads to the transition between these two stages, Mansfield *et al.* (2019) hypothesised that degradation of the plant cell wall by *H. fraxineus* may be activated by starvation. During this stage of infection, the authors observed that the plant cell walls became vacuolated and there was an increase cytoplasmic volume (Mansfield *et al.* 2019).

Initial symptoms of disease appear at approximately 12 days post-infection and are visible as necrotic lesions approximately 1-3 mm in diameter on the leaflets. The timing between infection and first symptom development varies, but this could be due to differences in the tissues tested and the methods used. For example, Mansfield *et al.* (2018) observed lesions at 7 days post inoculation on detached leaves and petioles, and 9 days post inoculation on internodes. However, Cleary *et al.* (2013) recorded a lag of 2 weeks between the first observation of apothecia on dead ash leaf petioles and the appearance of symptoms on the leaves of ash seedlings. During this phase of infection, the necrotic lesions spread along the leaflet lamina, leaf veins and petiole. Occasionally the pathogen then infects the stem (reviewed in Gross *et al.* 2014). However, stem infection is generally considered a dead end for the pathogen as sexual sporulation rarely occurs.

f. Overwintering

As *F. excelsior* is a deciduous tree, it sheds its leaves during autumn and these form part of the leaf litter. During autumn and winter, *H. fraxineus* produces a characteristic blackened pseudosclerotial plate that covers the petiole of the dead leaf. This structure enables

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H. fraxineus to overwinter by protecting the pathogen from factors such as desiccation and degradation by other microorganisms (Gross and Holdenreider, 2013; Kowalski *et al.*, 2013). In addition, the pseudosclerotial plate has been shown to enable the pathogen to delay sexual sporulation until the environmental conditions are favourable (Gross and Holdenrieder, 2013).

Research into the mating behaviour of *H. fraxineus* by Gross *et al.* (2012) demonstrated that the pathogen displays a heterothallic mating system, with strains exhibiting either the MAT1-1 or MAT1-2 idiomorph. Sexual reproduction can only occur between two opposite mating types and this leads to the formation of apothecia. Once conditions are favourable during the following spring/summer, the apothecia develop on the pseudosclerotial plate of the infected leaf petiole and the life cycle of *H. fraxineus* continues.

3.2 The *H. fraxineus* genome and virulence factors

Sequencing of the *H. farxineus* genome has furthered scientific understanding of the biology and evolution of this pathogen (e.g. Stenlid *et al.* 2017; McMullan *et al.* 2018; Elstrand *et al.* 2021). For example, McMullan *et al.* (2018) compared the genetic diversity of *H. fraxineus* isolates from across Europe and from a single wood in part of the pathogen's native range in Japan. Results from the research revealed a strong bottleneck of the European *H. fraxineus* population. This study also suggests that the European population was founded by two individuals, who may have come from the same site or even the same apothecia. A more recent study by Elfstrand *et al.* (2021) compared the genome of *H. fraxineus* against its non-pathogenic sister species, *Hymenoscyphus albidus*. Although the two genomes had high levels of synteny, the *H. albidus* genome has genomic signatures indicating that this species may be less able to adapt to a changing environment (Elfstrand *et al.* 2021).

Analysis of the *H. farxineus* genome has also elucidated genes with potential roles as virulence factors. For example, comparison of the *H. fraxineus* and *H. albidus* genomes by

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Stenlid *et al.* (2017) identified a high similarity in the ability to degrade plant cell walls. Both species have genes encoding Cell Wall Active Enzyme (CAZYme) profiles that are similar to that of saprotrophic fungi. However, *H. fraxineus* showed a higher gene expression of the two pectin-degrading enzymes, PL3 and GH 28. The activity of these two enzymes could result in the disruption of the plant primary cell wall, the fragments of which may act as elicitors of the host defence response (Stenlid *et al.*, 2017). In the same study, *H. fraxineus* expressed a short secreted protein (SSP) similar to the Cerato-Platanin (CP) family of proteins from known plant pathogens. The CP-like protein from *H. fraxineus* could act as an effector that may protect the fungal cell wall from enzymatic degradation by the host. Analysis of metallopeptidase expression by *H. fraxineus* and *H. albidus* during infection of *F. excelsior* demonstrated that *H. fraxineus* had higher expression of family M35 and M28A metallopeptidases. These metallopeptidases could be involved in the initial arms race between *H. fraxineus* and *F. excelsior*, such as inhibiting chitin-binding domain(CBD)-containing chitinases (Stenlid *et al.*, 2017).

In the study by McMullan *et al.* (2018), the authors identified 1,132 predicted secreted proteins that could serve as potential effectors. These include predicted secreted Cytochrome P450 proteins, which could have roles in breaking down ash-derived antifungal aromatic compounds, penetration and invasion of the ash tissue. Three effectors were also identified which have an NPP1 (necrosis-inducing Phytophthora protein) domain. This NPP1 domain is present in fungal, oomycete and bacterial proteins that induce hypersensitive-reaction-like cell death after infiltration *in planta*.

Previous research into the metabolites produced by *H. fraxineus* demonstrate that this pathogen produces the phytotoxin viridol and the secondary metabolite hymenosetin (Grad *et al.*, 2009; Andersson *et al.*, 2010; Halecker *et al.* 2014). Viridol has phytotoxicity to *F.*

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excelsior, and it can induce necrosis on ash shoots and lesions on seedlings (Grad *et al.*, 2009; Andersson *et al.*, 2010; Andersson *et al.* 2013). However, *H. albidus* also produces viridiol, and viridol concentration does not correlate with aggressiveness on detached leaves or seeds (Junker *et al.*, 2014). In addition, the putative biological gene cluster for viridol is conserved between *H. fraxineus* and *H. albidus* (Elfstrand *et al.* 2021). The specialised metabolite hymenosetin was isolated from virulent *H. fraxineus* strains by Halecker *et al.*, (2014), and the authors found that it has antimicrobial activity. This compound may therefore provide *H. fraxineus* with an advantage to capture substrate *in planta* by combatting competing microorganisms (Halecker *et al.*, 2014).

3.3 Interaction with the host

Ash trees display a range of susceptibility to *H. fraxineus*. For example, a study on *F. excelsior* clones in Austria across two years by Kirisits and Freinschlag (2012) found that ash dieback intensity ranged from no dieback to 80% dieback. Stockes *et al.* (2017) identified significant differences in susceptibility to ash dieback among provenances from the British Isles. Trees from middle Scotland were the least susceptible and may have been derived from a separate glacial refugium than the more susceptible populations from the South (Stocks *et al.*, 2017). The reasons for resistance to *H. fraxineus* may therefore be due to a combination of morphological and genetic characteristics.

Cytological analysis of infection of *H. fraxineus* by Mansfield *et al.* (2019) identified papillae-like deposits around penetration points, but these did not appear to affect fungal progress. However, a failed penetration attempt by the pathogen was association with localised changes to the outermost plant cell wall and ensheathment of small invading hyphae. Therefore, this could be a form of host response against *H. fraxineus* (Mansfield *et al.*, 2019). Comparison of ash genotypes has also elucidated metabolites associated with susceptibility

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and tolerance to ash dieback (Sambles *et* al., 2017; Sollars *et al.*, 2017; Nemesio-Gorriz *et al.*, 2020). For example, Nemesio-Gorriz *et al.* (2020) found a link between low susceptibility to ash dieback and increased levels of the coumarins fraxetin and esculetin. Both of these compounds inhibited *H. fraxineus* growth *in vitro* and the authors suggested their use as biomarkers to identifufy tolerant ash for selective breeding programmes (Nemesio-Gorriz *et al.*, 2020).

It is generally accepted that branch and trunk lesions are more likely to kill the tree than foliar lesions. Early leaf senescence could therefore be a protection mechanism against *H. fraxineus*, which prevents the pathogen from spreading through the foliage to bark and wood tissues. A study by McKinney *et al.* (2012) on disease symptoms of ash trees in Denmark across two years found a strong correlation between early leaf senescence and less infection of the shoots. In addition, McKinney *et al.* (2011) found that trees that flushing earlier were less susceptible to the pathogen. However, the study by Kirisits and Freinschlag (2012) did not find a correlation between the intensity of leaf shedding and dieback intensity. An additional study by McKinney *et al.* (2012) observed that healthier ash clones were able to limit the growth and spread of *H. fraxineus* following infection. Therefore, tolerance to ash dieback may be due to active defence mechanisms (e.g. cellular modifications, metabolite production) and as well as disease escape methods (e.g. early flushing, early leaf senescence).

4. Management techniques to control *H. fraxineus*

a. Prevention

Phytosanitary actions such as import bans, plant quarantine and diagnostic testing are key tools in preventing the introduction of plant pests and pathogens into new regions. Soon after the first detections of *H. fraxineus* in 2012, Ireland and Northern Ireland imposed bans on imports of *F. excelsior* from areas where the pest was present. Due to the widespread nature of the

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pathogen across the EU, and already existing bans on importing *F. excelsior* from outside of the EU, this in effect resulted in a ban on the import of ash plants and wood to the island of Ireland. This ban was later removed, after it was shown that the pathogen had become established on the island of Ireland and eradication was no longer achievable. If the outbreaks of the pathogen in Europe had been dealt with earlier, it is possible that the spread could have been contained. However, in the case of *H. fraxineus*, the uncertainty on the identity of the pathogen, the pathogen life cycle, and difficulties diagnosing infection contributed to the spread of the pathogen throughout Europe. Even after the comprehensive review on the biology and lifecycle of *H. fraxineus* was published in 2014 (Gross *et al.*, 2014), further major scientific breakthroughs were made such as the sequencing of the genome of the pathogen (Stenlid *et al.*, 2017; McMullan *et al.*, 2018; Elfstrand *et al.*, 2021), discoveries of the infective potential of the conidia (Fones *et al.*, 2016), and recording of a significant biotrophic stage in the infection cycle (Mansfield *et al.*, 2018). Such new discoveries are vital information so that effective legislative control measures can be implemented.

b. Sanitation

As detailed earlier, the ash dieback pathogen life cycle involves infection of the host each season by the pathogen. Therefore, curative treatments could have strong effects on managing the disease, if it is practical to apply such treatments. With seeds (van der Linde *et al.*, 2021) or young bare-root trees (Hauptman *et al.*, 2013), research has shown that the pathogen can be killed in plant material, and thus infection cured by hot water treatment. Immersion of ash saplings in hot water (36 – 40 °C) for between 5 and 10 hours was able to inhibit *H. fraxineus* and remove infection from the plants (Hauptman *et al.*, 2013). In nurseries it is particularly important to practice good biosecurity in order to prevent the spread of *H. fraxineus* and other pathogens among young plants. Biocides are a key tool for limiting spread,

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and a review by Cooke *et al.* (2013) hypothesised that biocides such as Salvox, Endosan, Endoquat and Envirocare could be effective in killing the pathogen in leaf material and on surfaces. However, the effect of these products against *H. fraxineus* still remains to be tested. Additional considerations for chemical control methods against the pathogen include timing of application and the efficacious concentration.

c. Removal of leaf litter

Ash leaves and fine woody material are the main matrix from which sporulation of the pathogen emerges (reviewed in Gross *et al.*, 2014). Therefore, any steps which lead to the reduction, or otherwise destruction of infected plant material will lead to reduced local spore production. Reducing spore production may slow local disease progression as high propagule build-up by *H. fraxineus* may enable the pathogen to overcome the host defence response (Cross *et al.*, 2017). At the tree level, removal of infected plants/trees will contribute to a reduction of spore loads as these plants will not shed infected leaf material. If infected leaf material is shed, its direct removal by mechanical means (e.g. raking) and composting (Noble *et al.*, 2019), or indirect removal by covering/burying (e.g. mulching) can lead to reduced spore levels being produced within the forest stand. Holb (2013) working in the *Blumeriella jaapii* (Rehm) Arx and cherry (*Prunus vulgaris* Mill.) pathosystem showed that removal of leaf litter as a single treatment, or in combination with straw mulching, led to significant reductions in the amounts of disease. Other novel techniques, such as speeding up the decay/destruction of the litter (e.g. by chemical means) or by livestock (e.g. sheep) are also hypothesised to have the effect of reducing local spore production (Figure 4; a). Research by Hauptman *et al.* (2015) demonstrated that urea applied at >2.5g/l significantly reduced *H. fraxineus* mycelial growth *in vitro* and decreased apothecia production from treated leaf litter. There is also evidence from other pathosystems that urea solutions can reduce sporulation of pathogens and are used in

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371 practice for treatment of apple and cherry leaves against the infections by *Venturia inaequalis*
372 (Cooke) G. Winter and *B. jaapii* in apple and cherry orchards (Sutton *et al.*, 2000; Green *et al.*,
373 2006).
374
375 **d. Planting in suitable sites**
376 There have been several papers focussed on silvicultural strategies to manage ash
377 dieback disease (Thomsen and Skovsgaard, 2012; Skovsgaard *et al.*, 2017; Short and Hawe,
378 2018) in recent years. These have provided guidance on suitable site selection, species
379 mixtures, and on forest management plans. For example, Marçais *et al.*, (2016) and Husson *et*
380 *al.*, (2012) found that site humidity is linked with canker severity.
381
382 **e. Planting mixed species**
383 In general, epidemiological theory indicates that mixed species forests suffer less from
384 disease at a stand level (Skovsgaard *et al.*, 2017). Evidence from another forest pathogen
385 (*Phytophthora ramorum*; Haas *et al.*, 2011), and across a number of pathogen examples
386 indicates that the presence of non-host plants can buffer susceptible trees against disease
387 (Prospero and Cleary, 2017). Skovsgaard *et al.* (2017) concluded that the effect of species
388 mixtures on disease levels needed more study, but highlighted that from a silvicultural point of
389 view mixed species forests allowed for the widest range of forest management actions to
390 modulate disease levels. Bartha *et al.* (2017) found that covering ash petioles with leaf litter
391 from other species promotes decomposition. In this study, ash petioles covered with *Tilia* leaf
392 litter had significantly higher fragmentation and decomposition rates than ash petioles that were
393 not covered. The authors hypothesised that covering ash leaves with leaf litter from other
394 species may promote maco-flora and micro-flora communities (Bartha *et al.* 2017). Thus, the

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effect of non-*Fraxinus* leaf litter, as a result of tree species mixtures, may also reduce the impact of *H. fraxineus* at the stand level.

f. Silviculture methods

Remedial silviculture strategies can be used to alleviate the impact of ash dieback and retain valuable trees that display tolerance to the disease. Short and Hawe (2018) have reviewed strategies, including case studies from Ireland, in detail. During the early stages of forest management, Skovsgaard *et al.* (2017) identified tending as the key point for managing the impact of ash dieback on productivity. Some of the key guidelines for tending centre on aiding the trees in avoiding the highly damaging trunk and collar lesions. Removal of low level branches, epicormic growth, and of lush vegetation around the base of the tree can help prevent these damaging collar lesions.

During the later stages of forest management, thinning can be carried out to (1) remove infected trees, (2) modify stand conditions and (3) reduce competition (Figure 4; b). Hygiene felling to remove infected trees will lead to a reduction in the amount of infected leaf material being produced in the site. This has been the primary method used in eradication attempts in Ireland and the UK. Thinning can also modify the stand environmental conditions, therefore reducing the spread of the pathogen. Skovsgaard *et al.* (2017) reviewed the results of several thinning experiments and found that trees in thinned plots are less damaged by the pathogen than those in un-thinned plots. Thinning can encourage airflow through the understory of the forest, which helps reduce pathogen sporulation and infection. Reduced shading on the forest floor can also cause temperature increases in the understory, and high temperatures have can negatively affect pathogen sporulation (Grosdidier *et al.*, 2018b). Finally, thinning can reduce competition between individual trees. This management technique therefore presumably allows ash trees to focus resources on defence against *H. fraxineus*. It is important to note that

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420 coppiced shoots are able to grow from stumps of felled ash trees, and these can become infected
421 by *H. fraxineus*. Therefore, preventing re-growth of shoots is required following thinning
422 (Cooke *et al.*, 2017).
423
424 Selection of diseased individuals for removal can be done via ground surveys
425 throughout the year. Surveys in the winter can identify stem and branch lesions, all of which
426 indicate previous infection and negatively affect timber quality. Surveys during the spring and
427 summer can identify crown dieback, due to the presence (or absence) of foliage on the tree.
428 Surveys in the spring-autumn are also useful to identify early flushing, or early senescing trees,
429 which, as discussed earlier, may be linked to lower ash dieback levels. A combination of
430 disease index and date of flushing senescence could be used to select for trees to retain. More
431 recently, spectroscopic imagery has been shown to have potential in discriminating between
432 ash dieback-susceptible and tolerant trees (Villari *et al.*, 2018), and its use in thinning
433 operations would be beneficial.
434 Resistance to ash dieback may be a heritable trait (Muñoz *et al.*, 2016), and so
435 protecting ash trees displaying tolerance to *H. fraxineus* is essential to prevent complete
436 extinction of the species (reviewed in McKinney *et al.*, 2014). Management practices, such as
437 thinning of diseased trees and the maintenance of healthy trees, therefore allows identification
438 of valuable seed resources to be collected for future use (Skovsgaard *et al.*, 2017; Short and
439 Hawe, 2018) (Figure 4; c).
440
441 **g. Treatment**
442 Fungicide treatment can be used to protect young trees in nurseries from *H. fraxineus*.
443 Previous research by Hauptman *et al.* (2015) demonstrated that carbendazim, prochloraz, and
444 chlorothalonil significantly reduce *H. fraxineus* mycelial growth *in vitro*. Of these three

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445 fungicides tested, carbendazim was the most effective as no fungal growth was observed at any
446 of the concentrations applied. In addition, carbendazim was the only fungicide which
447 completely prevented apothecia formation (Hauptman *et al.*, 2015). For larger trees the
448 application of fungicides is not generally feasible (DEFRA 2016). However, experimental
449 work using phosphite compounds have indicated that treated trees show increased survival
450 from the disease (Keča *et al.*, 2018). Another study has found that compounds containing
451 benzothiadiazoles, which incite the natural defences of ash tree, could prove useful in the
452 protection of ash plants from disease (Turczański *et al.*, 2021).
453
454 **h. Beneficial organisms and endophytes**
455 Biocontrol of *H. fraxineus* can be a method to reduce ash dieback in forest stands. For
456 example, integrated pest management strategies have shown promise for control of the apple
457 scab pathogen, *V. inaequalis* (de Jager and Heijne 2004; Holb *et al.*, 2006). Encouraging
458 beneficial microorganisms in ash forests may `promote the breakdown of ash leaf litter
459 (including the petioles) and reduce *H. fraxineus* inoculum. Endophytes can have a direct
460 antagonistic effect on plant pathogens by releasing toxic metabolites or competing for
461 nutrients. Alternatively, they may induce system resistance of the host. Therefore, these
462 beneficial microorganisms show promise as a method to protect ash trees. A number of studies
463 have identified endophyte species that reduce *H. fraxineus* growth or germination (Schlegel *et*
464 *al.*, 2016; Haňáčková *et al.*, 2017; Griffiths *et al.*, 2020; Halecker *et al.*, 2020). For example,
465 Halecker *et al.* (2020) identified the ascomycete fungus *Hypoxylon rubiginosum* that grows
466 asymptomatically *in planta* and produces the antifungal metabolite phomopsin, which inhibits
467 *H. fraxineus* growth *in vitro*. In addition, an investigation into the ash phyllosphere by Ulrich
468 *et al.* (2020) identified bacteria associated with tolerant and susceptible ash trees. Of these,
469 *Bacillus velezensis, Pantoea vagans,* and *Pseudomonas caspiana* were observed to be

antagonistic to *H. fraxineus*. The authors also suggested that non-antagonistic bacteria may be important biocontrol agents as these may act by indirectly activating the host immune system or providing niche competition against *H. fraxineus*. These studies therefore show promise for future strategies to develop beneficial endophytes that could be harnessed to protect ash trees from *H. fraxineus*.

5. Future Strategies for mitigation

There is a growing body of evidence of ways to mitigate the damage to *F. excelsior* from the pathogen *H. fraxineus*. Of the expanding literature, there are five topics in particular where scientific advances and intensive research effort could lead to important breakthroughs for ash dieback control and *F. excelsior* safeguarding. These are (1) continued phytosanitary actions to prevent further *H. fraxineus* introductions, (2) breeding of resistant *F. excelsior* trees, (3) identification of resistant *F. excelsior* trees, (4) harnessing the *Fraxinus* microbiome, and (5) the application of precision forestry.

5.1 Strengthened biosecurity to prevent further pest threats to *F. excelsior*

The spread of *H. fraxineus* across Europe between the 1990's to present day highlights the failures of the phytosanitary framework under the old plant health directive (2000/29/EC) to rapidly control a novel pathogen. It could be said that the legislation, bureaucracy and politics at the time did not respond in a suitable fashion to deal with the threat. Lessons have been learnt in many countries in the case of the response to ash dieback (Heuch, 2014; Tsouvalis, 2019). It is envisaged that the new EU Plant health regulation (EU 2016/2031) will allow for a more rapid response to unfamiliar or novel pest threats. What is clear from phytosanitary science is that preventing the introduction of a plant health pest is the only certain method of protecting plant health (Hansen, 2015; Liebhold *et al.*, 2016). It is also clear that the

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use of horizon scanning is key to provide an early warning of a pest threat. Detailed scientific study, leading to robust pest risk analyses enable countries to use the international phytosanitary framework to prevent the spread of a new pest into their jurisdiction (FAO 2016).

At the EU level, there is some justification for continuing to regulate *H. fraxineus* from outside of the EU. McMullan *et al.* (2018) showed that the *H. fraxineus* population in Europe had low genetic diversity compared to that present in the native range of the pathogen. This, they proposed, was a strong reason to prevent further introductions of the pathogen into Europe. Furthermore, comparison of virulence between European and Japanese *H. fraxineus* showed that the Japanese *H. fraxineus* is significantly longer lesions on *F. excelsior* (Gross and Sieber 2016). Regulating of non-European isolates of pests, even if those pests are already present in the EU, is currently practiced for pests such as *Phytophthora ramorum* and Tephritidae (aka fruit flies) under the EU plant health regulation (EU 2019/2072). Surveillance for the non-European populations of ash dieback would be vital to the implementation of any phytosanitary regulations aimed at preventing new *H. fraxineus* populations being introduced into Europe.

5.2 Genome enabled breeding for resistance

The sequencing of the *F. excelsior* genome and characterisation of genes inferring tolerance to *H. fraxineus* may shed light on targets for future selection and breeding strategies (Sollars *et al.*, 2017; Menkis *et al.*, 2019; Stocks *et al.*, 2019). The degree of damage caused by *H. fraxineus* can vary greatly and it is not uncommon to find single trees with little or no symptoms in a heavily infected areas. For example, a study in Denmark recorded between 1% - 69% damage and this was linked to high genetic variation (McKinney *et al.*, 2011). Resistance to ash dieback may therefore rely on the combined effect of a number of genes (qualitative resistance) rather than single resistance genes (quantitative) (McKinney *et al.*, 2011; Harper, *et al.*, 2016; Sollars *et al.*, 2017; Stocks *et al.*, 2019). Advanced genomic techniques are

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currently being used to map the genes of both the pathogen and the host, with the aim of boosting the ability of the host to defend against the pathogen. Genes for putative tolerance in *F. excelsior* have been identified by several studies (Harper *et al.*, 2016; Sollars *et al.*, 2017; Stocks *et al.*, 2019; Chaudhary *et al.*, 2020). In addition, the recent development of a protocol to transform *F. excelsior* calluses opens additional avenues to validate gene targets and study the molecular basis of ash dieback infection (Hebda *et al.*, 2021).

Tree breeding activities must be cognisant of not producing too homogenous host populations. In addition to *H. fraxineus*, the emerald ash borer (*Agrilus planipennis*) is a serious threat to Fraxinus. The emerald ash borer is present in North America, Russia and Eastern Ukraine, but it has not yet been detected in the EU (Valenta *et al.* 2015; Volkovitsh *et al.*, 2021). Comparison of ash genotypes and analysis of the *F. excelsior* genome suggests a link between susceptibility to ash dieback and high iridoid glycoside levels (Sambles *et al.*, 2017; Sollars *et al.*, 2017). Iridoid glycosides are compounds that have anti-herbivore activity among the Oleacea family. Therefore, there may be a trade-off between ash dieback susceptibility and herbivore susceptibility (including emerald ash borer susceptibility). In order to account for this, future breeding programmes will need to retain a high genetic diversity within the ash population to allow for flexibility in the selection and breeding of future ash trees (McKinney *et al.*, 2014).

5.3 Reliable and rapid identification of tolerant ash trees

Traditional methods to screen ash tree populations and identify tolerant individuals can be both time consuming and labour intensive. In addition, techniques such as marker-assisted selection have not been developed to the same extent for forest trees as they have for agricultural crops. Studies to date have identified possible disease tolerance markers using a combination of phenotypic characterisation, genotypic characterisation and associative transcriptomics

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(Harper *et al.*, 2016; Sollars *et al.*, 2017; Menkis *et al.*, 2019; Stocks *et al.*, 2019). For example, Menkis *et al.*, (2019) used phenotypic assessments and cDNA-based molecular markers to discriminate between tolerant and susceptible ash trees (Menkis *et al.*, 2019). As outlined previously, research by Villari *et al.* (2018) and Nemesio-Gorriz *et al.* (2020) used analysis of metabolite profiles to descriminate between tolerant and susceptible ash. The continued development of efficient tools to rapidly and reliably identify tolerant ash will therefore ehance efforts to breed for ash dieback-tolerant trees.

5.4 Harnessing the ash microbiome

Studies on the microbiome associated with *F. excelsior* and *H. fraxineus* can be used to inform novel strategies for control. As outlined in Griffiths *et al.* (2020), an understanding of the ash microbiome can be used to (1) select individuals based on microbial communities that are linked to host tolerance, (2) inhibit pathogen growth by inoculating with microbial mixtures, (3) alter the environmental conditions that promote a desirable microbiome, (4) genetically modify ash trees to impact signalling or selection traits that determine microbial community and composition (Griffiths *et al.*, 2020). Recent studies into the ash microbiome include comparisons between communities present on healthy and infected ash trees, comparing different ash tree tissues and analysing their temporal variation (Kowalski *et al.*, 2016; Cross *et al.*, 2017; Agostinelli *et al.*, 2021; Lahiri *et al.*, 2021). For example, the study by Griffiths *et al.* (2020) identified a significant association between severity of infection by *H. fraxineus* and the composition of the fungal and bacterial communities associated with ash leaves. In this study, the authors did not find a direct influence of host genotype on disease severity. However, the genotype did affect fungal community composition. Therefore, the authors suggested that host genotype could indirectly influence susceptibility to disease via genotype x microbiome interactions (Griffiths *et al.*, 2020). Further studies will elucidate the influence that *H. fraxineus*

570 has on ash microbial community structure, and this could inform future management strategies
571 against this pathogen.
572
573 **5.5 Precision forestry for optimised mitigation**
574 Precision agriculture is being used to increase the yield of crops, as well as reduce the
575 variability of produce and reduce input costs (Cisternas *et al.*, 2020). In precision forestry,
576 remote sensing and the use of radar technologies is enabling the management of the forest at
577 the stand, group and even tree level (Holopainen *et al.*, 2014). The use of such tools could
578 allow for rapid, low cost identification of diseased trees. Hyperspectral imaging has been
579 shown to be 77% accurate in assessing ash dieback severity (Chan *et al.*, 2021). From a disease
580 management point of view, these trees could be removed in order to stop the spread. The same
581 tools could also help identify disease tolerant trees, enabling forest management strategies that
582 aim to promote the health of these trees. When such remote sensing technologies are coupled
583 with tree growth modelling approaches, they may facilitate silvicultural decision-making
584 allowing forest productivity to be maximised. A barrier to the deployment of such precision
585 technologies for this purpose is the low monetary value of ash forests in Europe. However, as
586 the cost of such technologies, and the associated technical expertise becomes more economical,
587 it is possible that precision forestry will play a large part in the management of ash dieback and
588 other forest diseases.
589
590 **6. Conclusions**
591 Ash dieback disease has been one of the most damaging tree diseases in Europe. On
592 the island of Ireland the disease has generated high levels of public interest, as well as being a
593 major challenge for the forest industry in Ireland (McCracken *et al.*, 2017; Short and Hawe,
594 2018). While notable efforts are underway to protect *F. excelsior* from the disease, experience

595 in mainland Europe indicates that *F. excelsior* will become a rare tree in the Irish landscape in
596 the coming years. However, steps that can support and safeguard *F. excelsior* are not futile. It
597 may be through the protection of local stands of tolerant trees that the seeds of future resistant
598 trees are produced. One thing is certain, the fight against ash dieback disease will need to use
599 a variety of tactics if it is to be successful against this highly pathogenic and destructive
600 invasive fungal pathogen.
601

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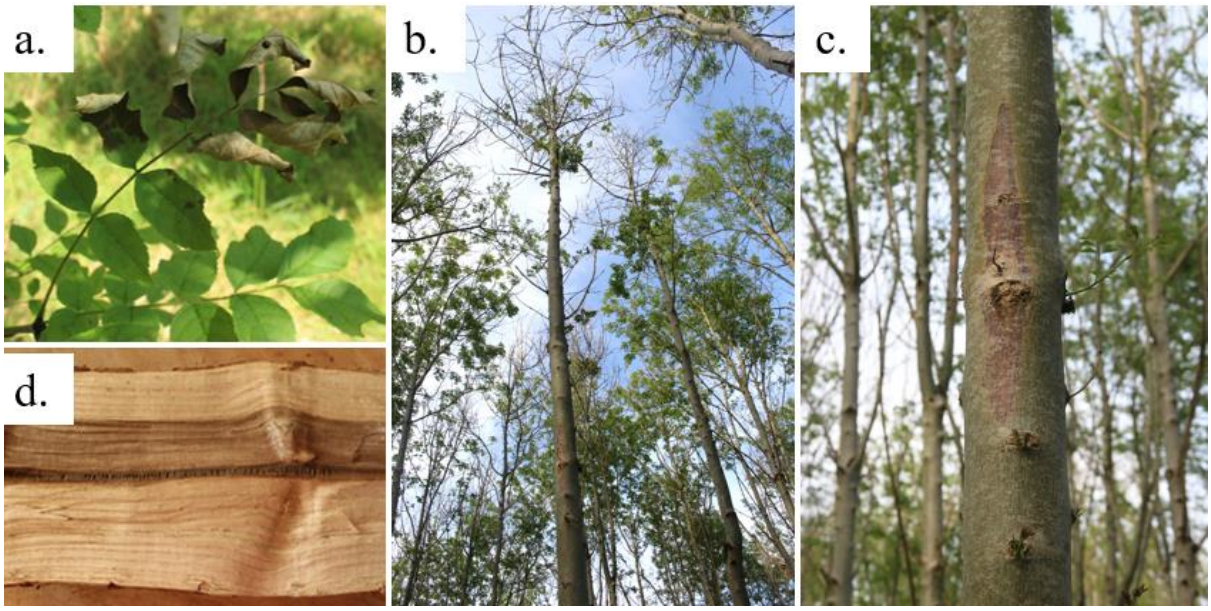
610
611 **8. Figures**



612

613 Figure 1: Images summarising the symptoms caused by infection of *H. fraxineus* on
614 *F. excelsior* at a forestry plantation in Co. Kildare. (a) dieback of at the tips of the leaves and
615 petiole; (b) crown dieback; (c) diamond-shaped lesion on the stem; (d) darkening of the
616 inside of stem. Images taken by A. Tiley (2021).

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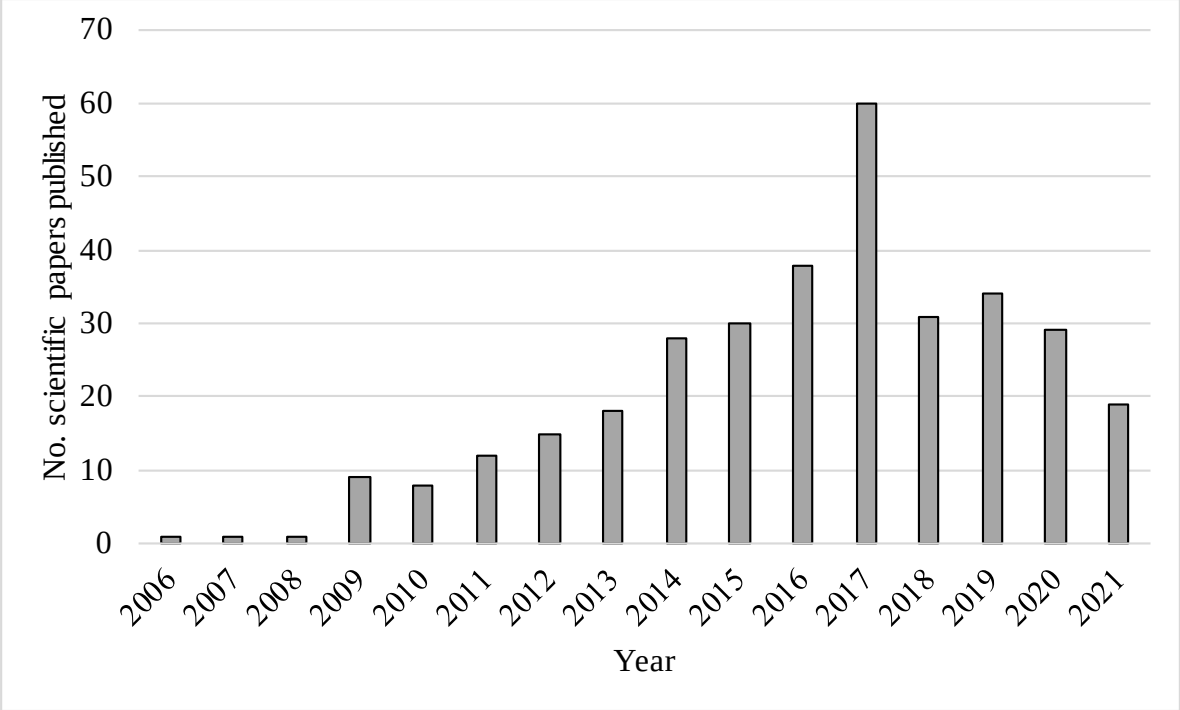

618

619 Figure 2: Number of peer reviewed scientific papers published on *Hymenoscyphus*
620 *fraxineus* (or its synonyms *Chalara fraxinea* or *Hymenoscyphus pseudoalbidus*) between
621 2006 and 2021 according to a Web of Science search (https://www.webofknowledge.com/,
622 last accessed January 2022).

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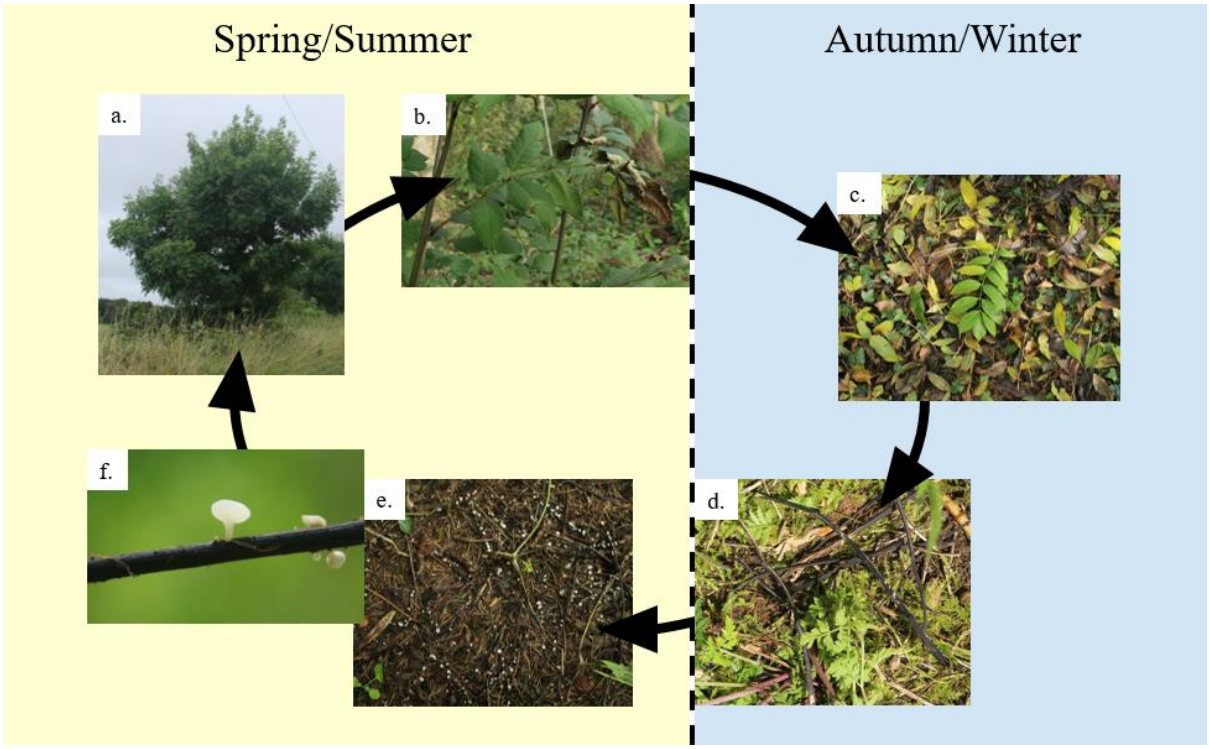

624

625 Figure 3: Images summarising the main stages of the sexual lifecycle of *H. fraxineus*
626 on ash trees. (a) During summer, healthy ash leaves are infected by wind-blown sexual
627 ascospores of *H. fraxineus*; (b) initial symptoms of infection include necrosis and wilting of
628 the ash leaves; (c) in autumn, the infected ash leaves fall to the ground and form part of the
629 leaf litter; (d) the leaflets degrade over autumn/winter and the fungus forms a black
630 melanised pseudosclerotial plate that covers the leaf petiole; (e) the following spring/summer
631 sexual fruiting bodies (apothecia) form on the infected petioles; (f) the apothecia release
632 sexual ascospores which continue the infection cycle. Images taken by A. Tiley (2021).

633

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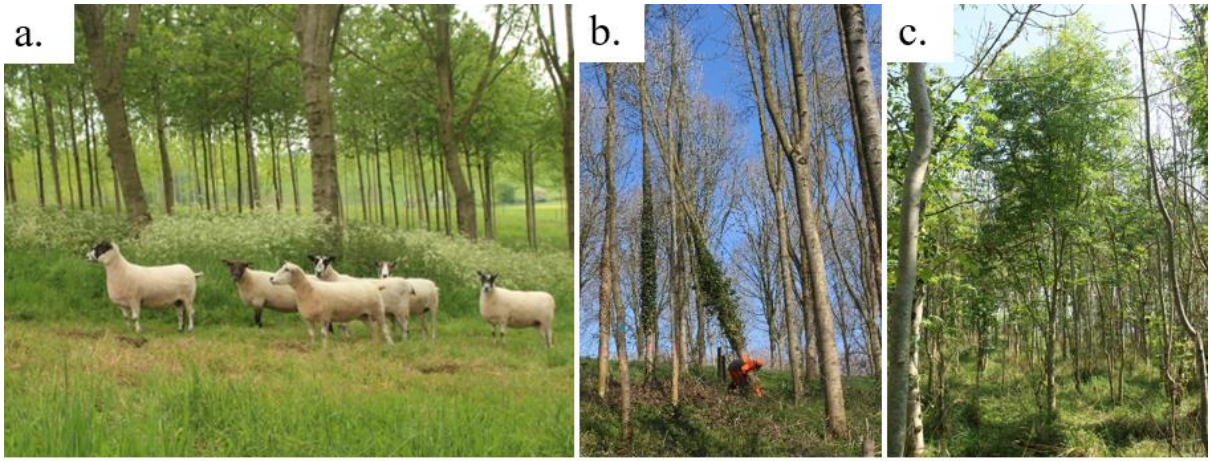
634 

635 Figure 4: Examples of local management strategies against ash dieback. (a)
636 experimental trials at to remove ash leaf litter by sheep grazing at AFBI Loughgall (Co.
637 Armagh); (b) thinning of infected trees at AFBI Loughall; (c) tolerant tree identified by
638 phenotyping among a heavily infected ash plantation, Co. Westmeath. Images taken by A.
639 Tiley (2021).

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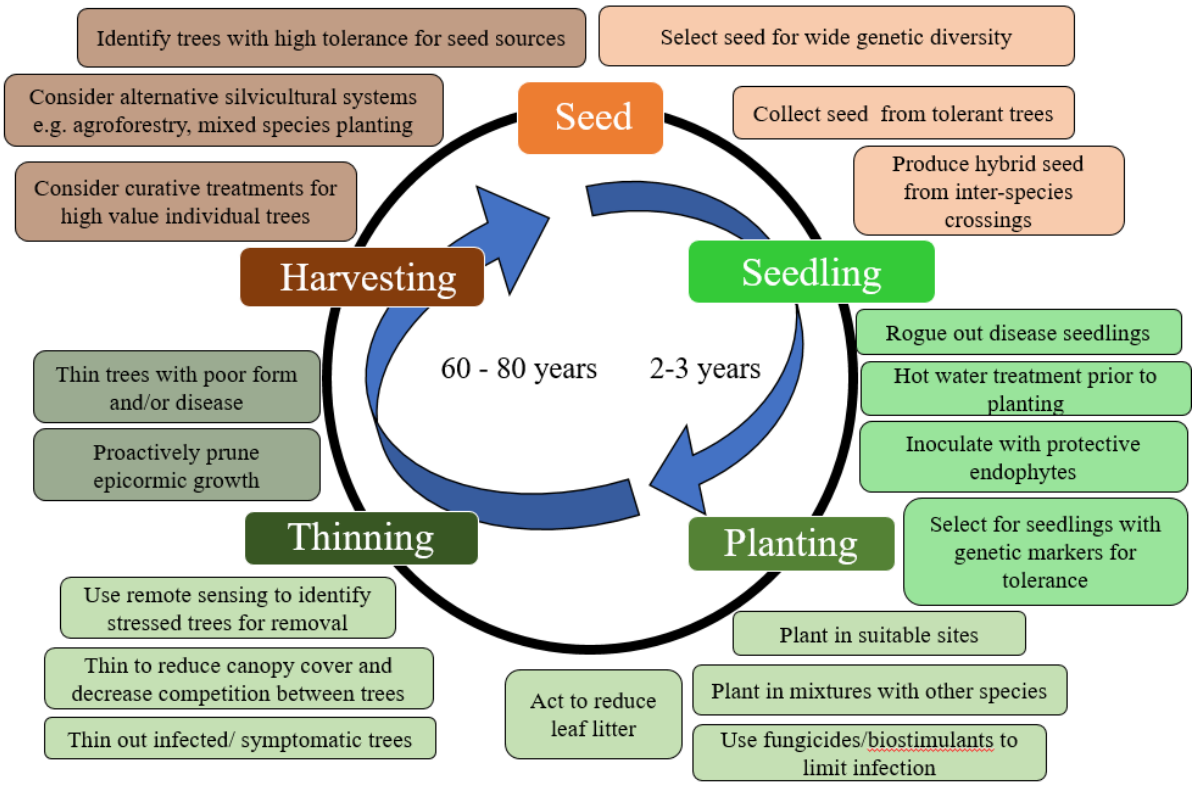

641

642 Figure 5: Schematic summarising potential ash dieback management strategies that

643 can be used at different stages in the lifecycle of an ash forest.

644

645

646

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