Agri-Food and Biosciences Institute ®

DARD E&I Project 12/3/S7

Efficacy of biocides, disinfectants and other treatments to limit the spread of ash dieback caused by *Chalara fraxinea*

Louise Cooke, Colin Fleming and Alistair McCracken
Sustainable Agri-Food Sciences Division, AFBI

www.afbini.gov.uk

DARD E&I project 12/3/S7: Efficacy of biocides, disinfectants and other treatments to limit the spread of ash dieback caused by *Chalara fraxinea*

Louise Cooke, Colin Fleming and Alistair McCracken
Sustainable Agri-Food Sciences Division, AFBI

Aims of work: A review of the available literature on disinfectants and biocides known to be/ having a high probability to be, effective against *Chalara fraxinea*. Development of best practice guidelines to optimise biosecurity measures used on-site, to safeguard against the spread of *C. fraxinea,* whilst complying with existing biosecurity protocols on disinfection to prevent the spread of notifiable animal diseases.

Scope: The routes for spread of the Ascomycete pathogen *Chalara fraxinea* (syn. *Hymenoscyphus pseudoalbidus*), the cause of ash dieback, from infected sites to uninfected ones will be considered. These will include:

- transfer of plant debris and spores on boots and other clothing
- transfer on wheels of vehicles and other equipment
- movement of infected plant material
- movement of wood.

Possible physical methods for removal and subsequent destruction of debris from machinery, equipment and footwear will be assessed. The available disinfectants, biocides and antifungal agents likely to be active against *C. fraxinea* will be reviewed for their suitability for use in biosecurity measures to prevent the spread of the pathogen by workers in and visitors to infected sites. In addition to products used throughout the world in agriculture, horticulture and forestry, this review will also include an assessment of potentially useful materials used for biosecurity in health care and laboratory safety. The relevant scientific literature will be consulted and availability, cost, safety and non-target effects will be considered. The existing biosecurity protocols for prevention of spread of notifiable animal diseases will be taken into account in the development of best practice guidelines. Where possible,

materials which will be effective against both plant and animal pathogens will be identified.

What are Biocides, Disinfectants and Anti-fungal Agents?

For the purposes of the present study on reducing ash dieback, the relevant products are biocides including disinfectants and anti-fungal agents including fungicides. These are covered by EU and UK legislation and any uses must comply with this. There are two main sets of regulations applying to this, the Biocides Products Regulations and the Control of Pesticides Regulations which specifically relates to pest control products including fungicides.

The Biocidal Products Regulations (BPR) and Biocidal Products Regulations (Northern Ireland) (BPR NI) implement a European-wide scheme (the Biocidal Products Directive 98/8/EEC) that covers a very diverse group of products, including disinfectants, pest control products and preservatives. These regulations control the use of biocides in the UK and are likely to apply to products used to reduce the spread of ash dieback.

A biocidal product is defined under the EU Biocidal Products Directive as “an active substance or a preparation containing one or more active substances, in the form in which it is supplied to the user, intended to destroy, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means.” or more simply a biocidal product is one which controls harmful or unwanted organisms through chemical or biological means. Harmful organisms include micro-organisms such as viruses, bacteria and fungi as well as insects and animals.

The Health and Safety Executive (the UK Competent Authority for biocides) assesses the safety of products containing biocides and the active substances within them to people, the environment and animals, and puts conditions on the use of these products. This is done through the Biocidal Products Regulations and the Control of Pesticides Regulations.

The legislation recognises four main groups of biocides (containing 23 different product types), these are:

- disinfectants, for home and industrial use
- preservatives, for manufactured and natural products
- pest control products
- other biocidal products, eg vertebrate control and other specialised products

Here we are concerned with primarily with disinfectants and also with pest control products. Chemical disinfectants can have various effects on organisms. Therefore, a basic understanding of the different chemical agents is important.

Biocide is a very general term referring to chemical agents that kill organisms. This general term includes disinfectants, antiseptics and antibiotics. Biocides generally react with proteins, specifically essential enzymes of microorganisms. Actions may include oxidation, hydrolysis, denaturation or substitution. The term biocide is commonly used to refer to agents with a broad spectrum of activity against a wide range of micro-organisms. More specific terms are used where only one type of organism is affected e.g. **Fungicide** refers to a chemical which kills fungi. Chemicals referred to as fungicides may be **fungistatic** rather than **fungicidal** in their action (i.e. inhibit growth rather than killing the fungus) and the term is also used for chemicals that inhibit *Phytophthora* spp. and other Oomycetes, although unlike *C. fraxinea*, these are not true fungi.

Disinfectant describes a product applied directly to destroy or irreversibly inactivate pathogenic microorganisms. These include fungi, bacteria and some viruses, but not necessarily spores.

Detergents serve to disperse and remove soil and organic material from surfaces allowing disinfectant to reach and destroy microbes within or beneath the dirt. These products also reduce surface tension and increase the penetrating ability of water, thereby allowing more organic matter to be removed from surfaces. Some detergents have disinfectant properties (e.g. quaternary ammonium compounds), so detergents may be used either alone or in conjunction with other disinfectants.

Detergents are classified in three categories: cationic, anionic and non-ionic.

Cationic detergents are positively charged solutions, and with the exception of quaternary ammonium compounds, are seldom used as cleaning ingredients.

Anionic detergents, or soaps, are negatively charged alkaline salts of fatty acids. They are less ideal for cleaning because they can be excessively foamy, creating a residue that may allow soil and microorganisms to accumulate.

Nonionic detergents are good emulsifiers, have good penetration and dispersion, are effective at lowering surface tension, and have reduced foaming properties. These products do not typically complex with metallic ions, such as those found in hard water.

Most commercial detergents are a combination of anionic and non-ionic.

In the context of preventing the spread of ash dieback, both chemical methods using biocides (specifically disinfectants, detergents and fungicides) and physical methods need to be considered.

All-Ireland Chalara Control Strategy (April 2013)

www.dardni.gov.uk/index/publications/pubs-dard-fisheries-farming-and-food/draft-all-ireland-chalara-control-strategy.htm

Objective 1 of the draft All-Ireland Chalara Control Strategy, developed jointly by DARD and DAFM in conjunction with AFBI and published 12 April 2013, is to reduce the risk of the disease becoming established in the wider environment. The aim of the present review of the available literature on disinfectants and biocides is to assist DARD in achieving that objective by developing best practice guidelines to optimise biosecurity measures used on-site and to safeguard against the spread of *C. fraxinea.* This includes proposing measures to be taken during surveillance, when dealing with recently planted infected trees and which could be used by DARD in its targeted advice and guidance to stakeholders and the general public. It is accepted that if Chalara ash dieback once becomes established in the wider environment, it is unlikely that Chalara ash dieback can be eradicated.

The Defra Chalara Management Plan (March 2013)

This review complements and expands on the approaches outlined in the Defra Chalara Management Plan (www.defra.gov.uk/publications/files/pb13936-chalara-management-plan-201303.pdf), published 26 March 2013. The plan notes that:

Treatment and prevention

Whilst there is currently no cure for Chalara, there are practical actions that that everyone can take and the Government remains open ideas for how the impact of Chalara can be tackled.

Leaf litter

The main source of Chalara spores is from fruiting bodies produced on overwintered leaves and released in the summer months. Circumstantial evidence from the continent suggests that trees in a woodland environment suffer more disease than trees in streets or parkland. This may be in part because more spores are produced from an undisturbed layer of woodland leaf litter than from ground which is swept, mown or grazed. We will work with landowners to identify possible sites where different approaches to management of leaf litter can be trialled and the speed and severity of damage from the disease compared.

Treatments

Based on our experience of other tree diseases, on scientific advice on and other European countries' experience of Chalara, we are advised against expecting to find a treatment which can be widely applied to protect woodland or treat an infected wood or forest. Treatments may have a role, though, in protecting individual trees or groups of trees, or reducing production of spores, level of damage and rate of spread in some circumstances.

Fera pro-actively sought potential products and various different chemical treatments have been proposed by companies or individuals. Those which show the most promise from the evidence available are now subject to laboratory and field trials. If the trial results indicate that one or more of the treatments could form an effective means of protecting such trees, the potential for extending the authorisations for the product(s) to cover relevant environments such as amenity or forest trees will be determined in consultation with the Chemicals Regulation Directorate of the Health & Safety Executive which regulates pesticide approvals.

The treatments that have been submitted for scientific analysis are now being taken forward as a matter of urgency to the next stage which is laboratory testing. These are a mixture of products which may be effective on live trees and those which may be effective on leaf litter. These products need to be tested to ensure they do not

adversely affect other wildlife or human health, and to ascertain how they might be used if appropriate.

In the USA, the New Pest Advisory Group (NPAG) has also stated that there is currently no information on effective controls for *C. fraxinea* (Appendix 1).

Before considering what measures are likely to be effective in reducing spread of *C. fraxinea* in the absence of specific information, it is necessary to outline the disease cycle in order to define the stages where intervention to prevent pathogen spread may be effective.

Ash dieback: cause and disease cycle

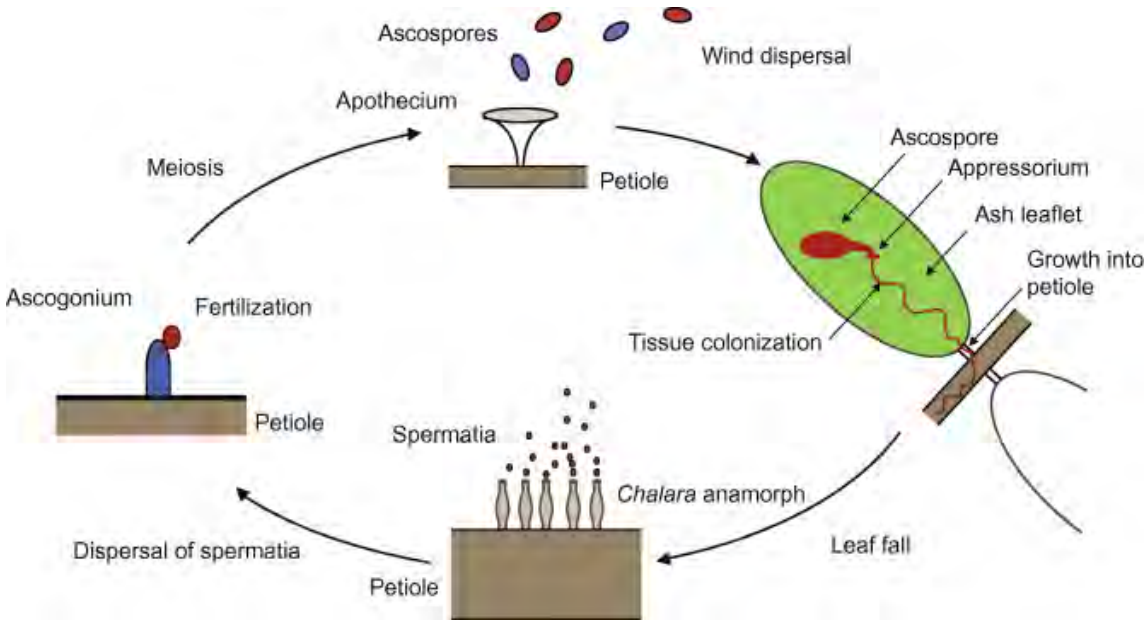
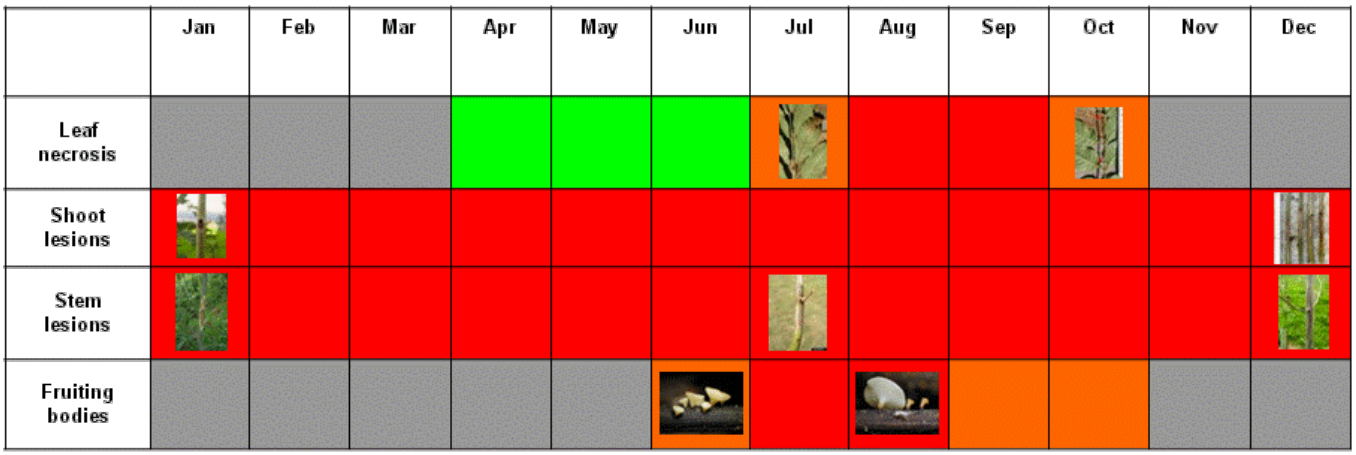


Figure 1. Hypothetical life cycle of *Hymenoscyphus pseudoalbidus*

Reprinted from *Fungal Genetics and Biology,* **49 (12)**, Gross, A., Zaffarino, P.L., Duo, A., Grunig, C.R. Reproductive mode and life cycle of the ash dieback pathogen. pp. 977–986, © 2012 with permission from Elsevier.

Figure 2. Schematic diagram of when symptoms are visible



Ash dieback is caused by the Ascomycete pathogen *Chalara fraxinea* (perfect stage *Hymenoscyphus pseudoalbidus*); for convenience it is referred to as *C. fraxinea* here. The hypothetical life cycle (Gross *et al.*, 2012, Figure 1) is based on the most recent scientific understanding of the pathogen, but this is developing all the time. The schematic diagram (Figure 2) shows when the various stages of the life cycle are believed to occur; it must be emphasised that this is extrapolated from what is known about the behaviour of ash dieback in other countries (mainly the Nordic countries and Eastern Europe).

Infection and spread within the tree

C. fraxinea infects ash trees through the leaves by air-borne ascospores (Cleary *et al.,* 2013b) produced on infected, fallen leaves during the summer months (July-August in mainland Europe) (Timmerman *et al.,* 2011). This results in the leaves withering and dying, and shoot lesions and stem lesions developing as the fungus grows into and through the leaf and into the woody tissue. Leaf death results from the death of the leaf stalks (petioles and rachises) and tree death may result as the trunk and branches are killed by the fungus growing through them. Ash dieback is not a vascular wilt like Dutch elm disease, where spores of the causal fungus spread through the tree in the sap stream and the tree reacts by blocking its vessels, killing itself as sap can no longer reach the twigs and branches. *C. fraxinea* grows through the woody tissue, killing it as it goes, into the heartwood of the tree (Schumacher *et al.,* 2010). Young trees with slender stems may therefore be killed quickly, but older trees may survive for several years, often succumbing to secondary organisms such as honey fungus.

Sporulation and spread between trees

As noted above, leaves infected by *C. fraxinea* die as the fungus spreads into the petiole and kills the stalks or twigs to which they are attached. They may be seen hanging on infected trees in the summer months (as they do not undergo normal abscission), but during the autumn and winter they become detached and fall onto the ground along with the other leaves. The leaves gradually decay in the leaf litter leaving just the petioles. In infected leaves, the fungus forms blackened structures, known as pseudosclerotia, in the petioles; these are simply melanised hyphae (strands of the fungus) which allow it to survive over the winter and in adverse conditions (Gross & Holdenrieder, 2013).

If both mating types of the fungus are present within the infected petioles (Bengtsson *et al.,* 2012) the fungus undergoes sexual reproduction and the following summer produces spore-bearing apothecia (Gross *et al.,* 2012). In adverse conditions (e.g. drought), the fungus can delay production of apothecia and survive for at least two years, producing apothecia in the subsequent summer (Gross & Holdenrieder, 2013). The apothecia release ascospores which become air-borne and infect ash trees via leaves completing the infection cycle.

Scientific opinion suggests that, although ash trees are infected through the leaves, if an infected tree survives for more than a year and leafs out in subsequent seasons, the new leaves may not themselves be infective (A. Gross, personal communication). This is because *C. fraxinea* may not grow back into them from the wood (depending on the proximity of lesions to the new shoot), although it would, of course, be possible for the new leaves to be externally infected by ascospores. If this is correct, it means that the main reason to remove and destroy infected trees is to get rid of the infected leaves in the first year of infection and that there is less advantage in removing larger infected trees that survive for more than a year. There is a lack of clear scientific evidence on this point: *C. fraxinea* is most frequently isolated from necrotic leaf stalks and necrotic bark from stem cankers; it has also been isolated from healthy looking leaf stalks, but the fungus in these could have originated from recent leaf infections rather than from growth of the pathogen out of wood or bark. Coppice shoots which develop on stumps soon after cutting can also become infected (Figure 3), and leaves falling from these may form pseudosclerotia over the winter and produce infective apothecia the following year (M.R. Cleary, SLU Sweden, personal communication). It is therefore important that cut stumps are treated with approved herbicides to prevent re-growth or that trees are completely grubbed out.

Figure 3. Shoots developing from a cut ash stump from a felled tree with ash dieback showing symptoms of infection by *Chalara fraxinea* (wilting).

Photograph courtesy of Michelle Cleary, Swedish University of Agricultural Sciences, Uppsala, Sweden.

There is no definite evidence that ash dieback can spread from tree to tree by any mechanism other than infection of ash leaves by *C. fraxinea* ascospores, and ascospores are produced only in apothecia which develop on leaf debris. Entry of ascospores into the tree at other points such as the collar is considered possible (Husson *et al.,* 2012), but would not have a major influence on control options. The maximum distance over which ascospores can be dispersed and still be capable of causing infection is not known although distances of between 30 km to over 100 km have been suggested. These estimates are based on modelling of ascospore dispersal (Anon., 2013) and on the spread of ash dieback symptoms [e.g. in Norway Solheim *et al.* (2011) reported annual spread of ash dieback as 20-30 km, while Worrell (2013) noted that in Sweden the disease spread across 900 km in 4 years]. Therefore the possibility that trees in Northern Ireland might be infected by ascospores blown across the Irish Sea from Scotland, Wales or England cannot be excluded. However, local spread from infected leaf debris is a far greater risk, since long-distance spore dispersal and infection will be a rare event.

C. fraxinea has been detected in felled ash wood on which it can produce asexual conidia, but so far it has not proved possible either to germinate the conidia or to demonstrate them to be infective (Husson *et al.,* 2012). Therefore while it is prudent to consider that the disease might be spread by movement of infected logs, the risk appears to be low and can be minimised by appropriate treatment and trimming.

In Sweden, *C. fraxinea* has also been detected at a low frequency in ash seeds produced by trees affected by ash dieback (Cleary *et al.,* 2013a). Whether infected seeds can play a role in the spread of ash dieback is uncertain, but importation of ash seeds from infected areas into Ireland is clearly a potential route of entry.

The potential introduction of *C. fraxinea* into Northern Ireland in infected plants, wood or seeds and movement of wood and trees within Northern Ireland is now subject to legislation [The Plant Health (Amendment No, 3) Order (Northern Ireland) 2012 and The Plant Health (Wood and Bark) (Amendment) Order (Northern Ireland) 2012].

It is clear from the disease cycle that the stage at which *C. fraxinea* is most dangerous in terms of disease spread, but when it is also most vulnerable to attack, is when it is in the leaf stalks in the leaf litter. Infective leaf stalks can be recognised by their blackened appearance (as opposed to the pale straw colour of normal leaf

stalks) and, during the summer months, by the presence of apothecia (1.5 – 3 mm), as shown in Figure 4. In addition, while not proven, it is possible that *C. fraxinea* might be spread during the process of sample collection if healthy trees were sampled after infected ones.

Figure 4. Apothecia of *Hymenoscyphus pseudoalbidus* (*Chalara fraxinea*) on infected ash petioles. Note the blackened appearance of the infected petioles, which contrasts with the pale colour of uninfected ones.

Photographs courtesy of Andrin Gross, Institut f. Integrative Biologie, Zurich, Switzerland.

Dangers of spread

The main ways in which *C. fraxinea* may potentially be spread are:

1. *in situ* production and release of ascospores from leaf debris at an infected site.
2. physical transfer of infected leaf debris to a new site followed by ascospore production and release.
3. infection of healthy trees by cutting them with contaminated equipment.

Once released, as noted above, the ascospores may move by air-borne spread anything from a few metres up to tens or even perhaps hundreds of kilometres, so preventing this occurring is vital to limiting the spread of ash dieback. There is thus a need both to prevent physical transfer of plant material and to render plant material, mainly leaf debris, non-infective.

1. By wind-blown spread of leaves
2. During sample collection and other work on sites by DARD or other personnel

2.1. On equipment used during sampling

2.2. On clothing particularly footwear

2.3. On vehicles (cars and bikes) particularly tyres

3. During public access to sites

3.1. On clothing particularly footwear

3.2. On vehicles particularly tyres

Physical methods to restrict the production and spread of spores (these may be used with or without the application of biocides and disinfectants).

1. **Removal and disposal of plant debris (which may contain apothecia) from infected sites infected sites or "isolation" of plant debris on an infected site.**

   1.1. Removal of leaf litter from infected sites

Effective removal and disposal of potentially infective (spore-generating) plant material, particularly old ash leaves, would be a very efficient way of reducing spore production and spread of the disease from *Chalara* positive sites.

Ease of removal of this material would be site-specific, but it would be possible to significantly eliminate much of the risk of spore production using this approach. Material would be collected and bagged for disposal. This approach would be best suited to small areas, without steep slopes and undergrowth or stones/rocks but even on optimal sites, it is unlikely 100% of infected material could be removed, so additional precautions may also be necessary.

Disposal of material collected from infected sites: The DEFRA advice note **Chalara dieback of ash - management of ash leaves and saplings (*Version 2.0, 6 December 2012*** www.forestry.gov.uk/forestry/infd-92gjvb) provides current guidance on the disposal of potentially infected material and the following options are suggested, in decreasing order of preference:

(a) Burning on site on the ground or in mobile incinerators brought to site (where these are used because they offer a practical solution to deal with a high volume of leaves);

(b) Burial in the ground (option for householders only);

(c) Composting on site;

(d) Incineration or landfill off-site; and

(e) Composting or other biological treatment off-site.

DEFRA note that "*there is no clear scientific evidence currently available on the effect of composting on Chalara spores. The temperature increase during the*

composting process, including anaerobic digestion and mechanical biological treatments, and the presence of decomposition fungi, which will decompose leaf material, rendering it unsuitable to sustain Chalara, might lead to its destruction. However, given the uncertainty, it is advised that wherever possible any resulting compost is spread on or near the infected source and not passed on to third parties who might transport it considerable distances for spreading elsewhere for agricultural or ecological benefit. Any leaves which are not destroyed or otherwise processed (e.g. through composting) should not be used for mulching or use on allotments where there is a likelihood of spreading the infection.

Burning is the preferred option where allowed under legislation on smoke control areas, and subject to the potential risk of smoke nuisance. The best way to do this is for householders, farmers and landowners to be considerate by advising their nearest neighbours before lighting a bonfire, so that they can be prepared for any minor inconveniences which might arise.

For local authorities and commercial landowners such as farmers, burial in land would constitute a landfill operation and would require an environmental permit which fulfilled the requirements of the Landfill Directive. For this reason local burial is not a practicable option. However, individuals acting in their own private capacity are not subject to the environmental permitting requirements, so householders may bury affected leaves within the curtilage of their premises if they wish.

Moving infected ash leaves for purposes other than destruction should be avoided where possible. Where it is not possible to deal with leaves from affected areas on site, the waste should be securely contained, either by bagging or by placing in enclosed containers and transporting the minimum distance possible for incineration (including energy recovery) or non-hazardous landfill at existing permitted facilities. Off-site composting and other biological treatment remains a less preferred option because of some uncertainty over the destruction of the fungus. Where the compost is to be used locally, this would mitigate against any possible residual risk".

It should be noted that while the DEFRA note refers to spores, to render infected ash material harmless, it would be necessary to achieve destruction of the melanised pseudosclerotia within the leaf petioles. As the pseudosclerotia are adapted as

survival structures, they are likely to be quite resistant to degradation by micro-organisms or destruction during composting.

1.2. Mulching

Another approach to reducing the production and spread of *Chalara* spores from infected sites would be to use a layer of mulch to cover these areas. Mulching with a layer of uncontaminated material (e.g. composted waste or pine needles) to a depth of 2-4 inches would cover infective ash leaves and restrict any spores from spreading to other areas. As with the physical removal of infected material, this approach would be best suited to smaller plantings, where the physical nature of the site allows easy access and movement of workers. However, there is no evidence as to whether mulching would kill the pseudosclerotia in the leaf petioles, and since it is known that these can survive adverse conditions for at least two years, there would be a risk that disturbance of the mulch could lead to the exposure of still infective material on which sporulation would then occur.

1.3. Covers and solarisation

Given the current evidence that spore production is restricted to the summer months, the potential use of artificial (e.g. plastic) sheeting could be considered. These types of materials, which are used to heat agricultural crops in early spring, would have the double benefit of changing the microclimate (i.e. solarisation/heating) of the soil surface (perhaps reducing spore production) and also preventing any spores that are produced from blowing away from the site to unaffected areas.

As with mulching, the size and physical nature of the site would greatly affect the effectiveness of this approach to spore control but it may be an option for small, flat easily accessible sites. The effects of covering and solarisation on spore production would need to be tested.

2. Preventing movement of potentially infected plant material to clean sites (footwear, machinery, equipment, bikes)

2.1 Restricted access

The movement of infective material by members of the public or staff working at infected sites is a likely significant source of new outbreaks. Perhaps the simplest way to avoid this is to restrict access to areas where infective ash leaves are known to be present. Assuming areas are well signed and “roped-off” this could be an effective approach. However leaves blown in the wind or transferred by wild or domestic animals means that the risk of spread of infected material is always present.

Where staff are working on multiple sites, good practice would be to schedule visits to infected sites after working completion of work at clean sites.

2.2 Brushes/mechanical removal from footwear, equipment and machinery

Where staff or public access is required/permitted, hygiene is a key step in the biosecurity of the site. Infected plant material can be carried on clothing, boots, equipment and vehicles. Physical removal of soil and plant material adhering to clothing and vehicles is therefore a high priority, before people, equipment and vehicles leave the infected site.

A wide range of static or rotating brush cleaners are available. They usually need to be used in conjunction with scrapers and disinfectant bath or sprays, but tend not to be very efficient (particularly when dealing with wet organic matter). If not managed properly, cleaning stations using brushes and scrapers can actually cross-contaminate footwear and equipment. Material removed at the cleaning station should be removed for disposal regularly.

It may also be required to use air blowers and or water hoses to help remove soil and plant debris from staff equipment and vehicles. In this situation it is important to ensure that wash off does not run into a watercourse or onto an uncontaminated area.

2.3 Air blower shoe cleaners

At sites with staff supervision and some infrastructure (e.g. electrical supply), it may be possible for members of the public to use shoe cleaning air blowers. A common feature of many golf clubs, these systems deal well with wet sticky plant material and soil and are designed to facilitate the collection of debris for removal and disposal (Figure 4).

There are many models available and most are electrically powered. They are generally low maintenance but are probably only suited to staffed sites and should be positioned beside permanent buildings where public and staff could use the facility on footwear or bicycles etc.

Figure 4. Typical air blower for cleaning shoes and equipment. Air jets transfer soil and plant material from shoes etc. into a collection bin for subsequent removal and disposal.

2.4 Preventing cross-contamination of cutting equipment

One of the risk factors which can contribute to *Chalara* spread is cross-contamination via cutting equipment. This primarily affects workers involved in the cutting and removal of infected or potentially infected ash trees, as well as those performing diagnostic sampling for the disease. In the latter case, as well as potentially spreading the disease, the high sensitivity of laboratory testing means that contaminated equipment such as secateurs could potentially give rise to false positive results.

Sterilisation of such cutting equipment is best achieved using high temperature flaming. While ideally ethanol should be used for flaming, the risk of burning ethanol being blown onto the user would be significant perhaps even leading to clothing catching fire or ignition of the ethanol source. For this reason we would recommend that the field sterilisation of equipment should comprise:

- Cleaning any organic matter and residue from the blade with a wet wipe or similar (i.e. a wipe impregnated with ethanol or biocide which should be retained in a bag or container for proper disposal, biocide-impregnated wipes are available from several manufacturers) before flaming

- Moving the blade through a hot flame (generated by a kitchen blow torch or similar source), ensuring that each area of the blade has been flamed for 2-3 seconds.

Chemical methods using disinfectants and fungicides

Information on the use of chemicals in the following sections is provided for guidance only and no liability is accepted for any error or omission in the content, nor for any loss or damage arising from the use of the products mentioned. It is essential to follow instructions for use of products and, in particular, to avoid the use of chemicals near water courses to prevent contamination of waterways.

1. Biocides/Disinfectants for treatment of contaminated footwear, clothing and equipment

1.1. For use by DARD and other professional staff

A wide range of disinfectants is available and some are in use to prevent spread of plant and animal diseases. Sources consulted included the DARD Veterinary Service, DARD Agri-food Inspection Branch, the Forestry Commission and Defra. Fera kindly provided a list of products suggested by those who had responded to their call to provide information on possible options for ash dieback control. This included a number of biocides and was examined critically for those which might be effective. Those considered in detail are listed in Appendix 2.

The products used by DARD Veterinary Service for prevention of spread of animal diseases are based on iodophors and acids and are primarily targeted against bacterial pathogens. Iodine has antifungal activity, but iodophors are less active against some fungi (McDonnell & Russell, 1999) and as they are potentially irritant and corrosive, they are not considered appropriate for use as ash dieback biocides.

Afib and the Forestry Commission both use alcohol-based products (Klercide, Propellar) for routine disinfection (ethanol and isopropanol, respectively). Alcohols are effective in killing fungal pathogens and the sprays are straightforward to use. The downside is that they are inflammable and expensive (the products used are primarily for medical use so are likely to be manufactured to a very high standard of purity, which is not actually required for ash dieback biocides). In APSBD laboratories in AFBI, Mikrozid is used, which is a similar product (contains ethanol and propanol), with the same drawbacks.

Afib use Cleankill for general disinfection. This product contains several bactericides including benzalkonium chloride and didecyl dimethyl ammonium chloride. These compounds are known to be active against *Phytophthora ramorum* (James *et al.*, 2012) and *P. cinnamomi* and have also been shown to be active against the fungi *Mucor amphibium* and *Batrachochytrium dendrobatidis*, which cause diseases of amphibians (Webb *et al.,* 2012). While *M. amphibium* and *B. dendrobatidis* are only very distantly related to the Ascomycete fungi, it is likely that they are active against Acomycetes such as *C. fraxinea* although this does not appear to have been tested.

Of the other biocides/disinfectants, Jet 5 and Sorgene 5 are both known to be active against Ascomycete plant pathogens (Clayton *et al.,* 2001) and are recommended by AFBI and CAFRE (as well as by the Potato Council and SRUC) for disinfection of potato stores. These materials are corrosive and oxidising so would require use of protective clothing and might damage contaminated vehicles or equipment.

Only four of the biocides in the list of products suggested to Fera appeared of potential interest. Salvox is a hypochlorous acid formulation; sodium hypochlorite solutions (household bleach), which produce hypochlorous acid, are widely used as surface disinfectants including in plant pathology to kill contaminating micro-organisms, so would be effective, but are skin irritants. However, Salvox is said to be said to be non-irritating and skin-safe, so this product may be suitable for decontaminating equipment and clothing. Endosan (silver stabilised hydrogen peroxide) is combustible and irritant, but its manufacturer reports activity against 'mould' and suggests use combined with their quaternary ammonium Endoquat. The other two, Endoquat and Envirocair, are both quaternary ammonium compounds and are also non-irritant and skin-safe. Both of these are said to be active against fungi and Envirocair specifically mentions *C. fraxinea,* but it appears that this is based on extrapolation from activity against fungal human pathogens. Nonetheless, quaternary ammonium compounds are known to be active against fungal plant pathogens and would also be a very safe option, so should be considered particularly for use in locations with public access. The manufacturers of Endoquat and Envirocair have provided additional information and expressed interest in working with us to determine effectiveness and methods of use.

1.2. For use by the public at infected sites

The main risk in terms of public access to infected sites is physical transfer of infected leaf debris to uninfected sites on footwear. Footbaths for disinfection of footwear could be provided. If this were to be done, then the quaternary ammonium products noted above (Endocair or Endoquat) should be assessed for their suitability and effectiveness.

2. Fungicides, biocides and other products for treatment of infected leaf debris

Treatment of infected leaf debris to kill *C. fraxinea* before it can produce ascospores appears a superficially attractive proposition, but in practice is fraught with problems in terms of logistics and legalities. It is probably only feasible in specific locations where infected debris has been identified (blackened leaf stalks) and occurs in a defined area. The widespread application of biocides or fungicides to the environment could potentially have undesirable side-effects on other organisms such as earthworms and lead to a build-up of chemical residues which might contaminate water courses. However, there may be some situations where chemical treatment could be appropriate, although, even then, it should be supplemented with physical approaches.

To be effective, a chemical treatment would need to do one or more of the following:

- kill *C. fraxinea* pseudosclerotia
- prevent apothecial formation or ascospore maturation by promoting rapid leaf breakdown
- kill the ascospores within the apothecia
- prevent ascospore release.

2.1. Fungicidal treatment of leaf debris

Apart from environmental considerations, there are two major problems associated with fungicidal treatment of leaf debris:

- the melanised pseudosclerotia are adapted as survival structures with thickened hyphal walls so are likely to be resistant to treatment with fungicides

- no fungicides are legally approved for application to leaf debris for preventing spread of ash dieback so any such use would require approval of the Chemicals Regulation Directorate (CRD).

There is no published information on efficacy of fungicides against *C. fraxinea.* Although there is some published research on control of *C. elegans* (Labuschagne & Kotzé, 1996), which causes blackhull of groundnuts, this is a very different disease and many of the fungicides evaluated are no longer available.

Some fungicides have off-label approval for use in amenity vegetation or forestry (Appendix 3), but this would not permit their use against ash dieback. As there is no information on their likely efficacy, they would need to be tested before their use could be considered. It has been suggested that products approved for control of apple scab might be active. This is because the causal fungus, *Venturia inaequalis*, is an Ascomycete with a disease cycle with some similarities to that of ash dieback, including production of ascospores on infected, fallen leaves in the spring and summer. Fungicides approved for apple scab control are listed (Appendix 4). Products active against apple canker are also worth consideration.

If it were acceptable to spray leaf litter with fungicides, then benzimidazole fungicides (carbendazim, thiabendazole) should be evaluated as they are persistent and anti-sporulant (carbendazim is effective in reducing apple canker when applied as foliar sprays to protect leaf scars and other entry points; Cooke, 1999). However, they lost approval for use on apples some years ago (although are still approved for use on cereals) and have adverse effects on earthworms.

Copper fungicides have been proposed, but these are potentially toxic to the environment and to trees (they are used to prevent leaf-scar infection by apple canker but this is an autumn treatment during leaf-fall with only two applications per season; Cooke, 1999).

Other fungicides may be worth evaluating, however efficacy against the pathogen *in vitro* carries no guarantee of effectiveness in preventing sporulation from fallen leaves *in vivo.* Fungicides may not penetrate and kill the pseudosclerotia, so that persistent activity would be required to prevent production of apothecia or to kill ascospores as they are released. In addition, many fungicides are readily bound to soil so might not be biologically available and regular application would be required if

leaf-fall were ongoing. Fera are evaluating selected fungicides for activity against *C. fraxinea* (Appendix 5) and are starting with laboratory screening and it is recommended that AFBI continues to liaise with the scientists involved in this work.

We question the environmental acceptability and effectiveness of attempting fungicidal treatment of leaf debris which would require regular application of persistent fungicides.

2.2. Biocides and other products for treatment of leaf debris

Urea is a possible option as it is used against apple scab to promote breakdown of the fallen leaves over the winter period preventing survival of ascospores in leaf litter and subsequent spore release the following spring (MacAntSaoir *et al.,* 2010).

Seán MacAntSaoir (AFBI Loughgall) has stated "As an autumn fertiliser (to strengthen the apple buds) we apply 5 kg/ ha. For leaf decomposition we use 2 kg urea/ha. A conventional orchard sprayer would be suitable to spray hedge rows as long as drift is not an issue". This might be effective and acceptable in some situations.

There is a lack of clarity regarding whether use of urea in this way requires specific approval under Pesticides Regulations since it may be considered that application to encourage leaf breakdown is not use as a pesticide. However, CRD advise:

"Authorisation for urea in the UK exists only as an individual tree stump fungicide treatment. See link for further details:
http://www.pesticides.gov.uk/guidance/industries/pesticides/topics/pesticide-approvals/commodity-substances/commodity-substance-urea.htm

Use as a leaf litter degradation spray (with the specific aim of assisting in the control of ash dieback) would be outside of this and would probably need a separate authorisation." "Urea does have Annex 1 listing and there is a published EFSA conclusion on it so it would be possible for CRD to conduct a risk assessment if a dose can be established."

Further advice would therefore be needed if such use were to be advocated (Fera may seek this, since urea is on their list of options for evaluation, Appendix 5).

Surfactants are another possible option for treatment of leaf debris, which might kill over-wintering *C. fraxinea*. In the 1970s, dormant season treatment of apple trees with surfactants was shown to eradicate apple mildew (caused by the Ascomycete fungus *Podosphaera leucotricha*) by killing the pathogen within infected buds (e.g. Clifford *et al.*, 1981) and ICI Plant Protection marketed one of these, a non-ionic surfactant coded PP222, as Dormakil. The active material was able to penetrate through the bud scales in order to kill the pathogen. Burchill & Swait (1977) also showed that some surfactants could eradicate the perithecial stage of apple scab, *V. inaequalis*, within infected leaves. Although the surfactant chosen by ICI for development was non-ionic, research at Long Ashton Research Station showed that other types of surfactants including quaternary ammonium compounds were also very effective as apple mildew eradicants. Dormakil was eventually withdrawn as more active fungicides became available and because if the apple trees were not completely dormant when it was applied, it could cause phytotoxic damage. However, this work suggests that, as well as having potential for use as biocides for treatment of contaminated equipment and clothing, surfactants, particularly quaternary ammonium compounds, should be considered as options for treatment of leaf debris. The environmental acceptability of such treatment would need to be examined as some surfactants are toxic to other organisms such as earthworms. Even if direct application of surfactants to leaf debris on the ground were unacceptable, the possible eradicant effect on the fungus in debris picked up on footwear makes their use for treating contaminated clothing of greater interest.

Biologicals: use of plant extracts or other biological materials that might encourage leaf breakdown and/or inhibit sporulation would be a more environmentally acceptable option. One such option is Ecospray, a garlic extract, which is known to be biologically active against a range of organisms and is being evaluated by AFBI. The use of other micro-organisms such as native *Hymenoscyphus* spp. to promote leaf breakdown or preferentially colonise leaf debris is also an option, but one which would require research. This is analogous to the use of *Phlebiopsis gigantea* in forestry where its application to cut pine stumps prevents their colonisation by the pathogenic fungus *Heterobasidion annosum* which otherwise infects them and then spreads to healthy trees (Greig, 1976).

3. Fungicides and other products for treatment of trees

Treatment of trees is outside the scope of this review, but it is considered worth including a brief comment on this as DARD is likely to be asked about feasibility. There are two options: prophylactic or protectant treatment to prevent healthy trees becoming infected and therapeutic or eradicant treatment of infected trees.

3.1. Protectant treatments

As noted above, Fera will screen selected fungicides which represent a wide range of modes of action against *C. fraxinea* in the laboratory and will then test those which show activity *in vitro* as protectant treatments on ash seedlings in spring 2013 (Appendix 5), so this will provide data on efficacy. Updates on the results of this work will be published on the Fera website at www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/chalaraInfo.cfm.

Since *C. fraxinea* infects trees through the leaves, prophylactic fungicide applications would need to protect the leaves in the same way that fungicides for the control of apple scab are applied to trees in orchards using mist blowers. Thus the fungal spores are killed on the leaf surface before they can penetrate the tissue. While it is quite likely that some fungicides might be effective in protecting ash trees from dieback if applied in this way, maintaining protection would require a regular programme of applications throughout the period of risk (i.e. while ascospores are being released), since most fungicides do not persist for long periods (this being environmentally unacceptable), which would need to be continued every year while the risk remained. This might, perhaps, be feasible for protection of an individual high-value tree (but the larger the tree, the more difficult it is to achieve good spray cover), but would not be acceptable for trees in the countryside in natural woodlands and hedgerows. Achieving protection by applying fungicides as root drenches is unlikely to be effective: uptake would be limited (many fungicides bind to soil), so that a biologically effective dose in the leaves would not be achieved and drenching soil would be as environmentally unacceptable as foliar sprays. It is possible that advanced trunk injection technologies being developed by Syngenta (personal communication) might be of use, but it would be very challenging to maintain a dose in leaves sufficient to give protection for several months and again annual treatment would be required.

Scientists at Imperial College, London's Silwood Park Campus are evaluating a patent copper sulphate solution, CuPC33, developed by Natural Ecology Mitigation. A press release of 7 November 2012 stated "*The scientists say that CuPC33 could be dispersed through infected woodlands by spraying or as a dense medicated mist that lands on leaves and branches. Using technology that atomizes the liquid into very tiny droplets, they anticipate that ten litres of diluted CuPC33 is sufficient to treat one hectare of forest at a material cost of less than 60 p per litre*." Natural Ecology Mitigation consider that CuPC33 will be effective in controlling *C. fraxinea* and hope to carry out field trials in spring 2013. However, to date there is no published evidence to support the use of this product nor does it have UK approval as a fungicide. Products containing copper oxychloride and Bordeaux Mixture (a complex of copper sulphate and calcium hydroxide) are approved as fungicides for control of a range of diseases including of trees and, as noted above, copper oxychloride can be applied to apple trees at leaf-fall in the autumn to protect leaf scars from infection by *Nectria galligena,* the cause of canker. However, copper fungicides are protectant and non-systemic, there are strict limits on the amount of product which can be applied and they can cause phytotoxic damage to plants if applied in the growing season.

3.2. Eradicant treatments

It is widely accepted that treatment of infected trees is most unlikely to be effective. Once inside the tree, *C. fraxinea* grows into the heartwood and attempts to treat it will be very problematic. It would be necessary to use specific systemic fungicides i.e. single-site inhibitors active against Ascomycete fungi. Introducing broad spectrum fungicides or biocides into trees is likely to cause severe phytotoxic damage if they are injected, while if they are applied as sprays or root drenches, they will simply to fail to be taken up by the tree. While there has been limited success in treating vascular wilts such as Dutch elm disease using trunk injection, in ash dieback the fungus is not in the vascular system so reaching it with a fungicidal dose would be difficult. In addition, even if effective, trunk injection would need to be repeated annually as long as the threat of infection remained, so is only suited to large, high value trees. Applying systemic fungicides as sprays or root drenches is unlikely to achieve sufficient uptake to kill the pathogen within infected trees

(systemic fungicides move acropetally in xylem, so spraying leaves will not result in downwards translocation into the tree to any significant extent).

For these reasons, and since the Northern Ireland policy is one of eradicating ash dieback, therapeutic treatment of infected trees or prophylactic treatment of healthy ones will not be considered further here.

Conclusions from the review and best practice

The following recommendations should not be considered definitive. Ongoing R&D and tests across Europe may deliver new evidence leading to changes in recommended methods for *Chalara* control.

While the long distance spread of ash dieback can be linked to trade and movement of ash trees, current knowledge suggests that in the environment, fallen ash leaves are the major source of disease re-infection and spread via spore production.

- Removal of leaf debris from infected sites would greatly reduce the subsequent production of apothecia and spores and should deliver a major reduction in the likelihood of disease spread. Physical removal of ash leaves may only be possible on smaller sites and where the physical conditions permit easy access for workers and collection of leaves (e.g. flatter sites, low levels of undergrowth etc). Where this is possible, leaves should be bagged as they are collected and bags sealed and removed for disposal.
- Where leaf removal is not possible, promoting the breakdown of ash leaves will have a positive effect in preventing sporulation. Application of urea in winter/spring to stimulate breakdown of the leaf litter could be beneficial, but may raise regulatory issues (Fera is testing this approach so liaising with them is recommended).
- Spraying leaf litter to kill the pseudosclerotial stage of the fungus in the leaf debris with quaternary ammonium biocides may be effective (testing is required). Fungicides are not recommended at this stage.
- If sites actively producing apothecia and spores are identified, there will be an urgent need to destroy the fruiting bodies and spores. Current evidence would support the physical removal of leaf litter and/or the use of sprays to kill the fungus. Surfactants may be effective in this respect but this method would require testing. Similarly, benzimidazole fungicides could have a role in the rapid treatment of infected sites but their use would require CRD approval and would present environmental issues.
- Biosecurity measures at infected sites can have a major impact on disease spread. The use of access restrictions and proper signage can prevent the

transfer of infected material onto footwear, tyres and equipment, but infected sites also need to implement cleaning stations at exits, especially for footwear and wheels.

- Where the public and workers are accessing infected sites, boot cleaners (brushes and/or air blowers) can be most effective. In addition, footbaths and wheel washing stations using quaternary ammonium biocides are recommended for restricting the transfer of *Chalara* to other sites.
- Particular attention should be paid to the biosecurity measures used by workers undertaking diagnostic sampling for *Chalara*. All of the above methods for preventing the transfer of organic material on footwear, wheels and equipment should be used, as well as the flaming of sampling equipment and the use of disposable gloves and/or handwipes and sprays.
- DEFRA are currently undertaking evaluations of a number of fungicides and other materials for *Chalara* control. The results of these tests could have a significant effect on modifying best practice recommendations.

Recommendations for further work

- Evaluate the activity of selected biocides, notably quaternary ammonium compounds, and of Ecospray garlic extract against *C. fraxinea,* for:
    - their effectiveness in killing pseudosclerotia within ash petioles
    - their effectiveness in killing apothecia and/or ascospores
- Assess methods of applying biocides active against *C. fraxinea* to leaf litter.
- Follow up contacts with biocide manufacturers to determine the most appropriate formulations, the formats in which they can be supplied (bulk, sprays, wipes) and the costs associated with these. Both Biotech International (manufacturer of Envirocair) and Endoenterprises (manufacturer of Endoquat and Endosan) are interested in collaboration.

Acknowledgements

The authors acknowledge the help of many colleagues within AFBI, DARD and elsewhere in providing information used in this review. They particularly thank Fera for providing details of responses to their call for potential control measures for *C. fraxinea*, Chemicals Regulation Directorate for regulatory information and Andrin Gross, Institut f. Integrative Biologie, Zurich, Switzerland and Michelle Cleary, Swedish University of Agricultural Sciences, Uppsala, Sweden for their helpful comments on the epidemiology of *C. fraxinea.*

References

Many papers on the biology and epidemiology of *Chalara fraxinea/Hymenoscyphus pseudoalbidus* have been consulted, but only selected papers are listed here.

Anon. (2013). Chalara Management Plan, Defra, www.defra.gov.uk/publications/files/pb13936-chalara-management-plan-201303.pdf 34 pp.

Anon. (2013). All-Ireland Chalara Control Strategy, DARD, DAFM, draft 12 April 2013, www.dardni.gov.uk/index/publications/pubs-dard-fisheries-farming-and-food/draft-all-ireland-chalara-control-strategy.htm 17 pp.

Bengtsson, S.B.K., Vasaitis, R., Kirisits, T., Solheim, H., Stenlid, J. (2012). Population structure of *Hymenoscyphus pseudoalbidus* and its genetic relation to *Hymenoscyphus albidus. Fungal Ecology* **5**, 147-153.

Burchill, R.T., Swait, A.A.J. (1977). Eradication of the perithecial stage of apple scab with surfactants. *Annals of Applied Biology* **87**, 229-231.

Clayton, R., Wale, S.J., Blackwood, J.M. & Black, S. (2001). *Potato store hygiene and disinfection to improve seed health and ware quality* BPC project report number 2001/5.

Cleary, M.R., Arhipova, N., Gaitnieks, T., Stenlid, J., Vasaitis, R. (2013a). Natural infection of *Fraxinus excelsior* seeds by *Chalara fraxinea. Forest Pathology* **43**, 83-85.

Cleary, M.R., Daniel, G., Stenlid, J. (2013b). Light and scanning electron microscopy studies of the early infection stages of *Hymenoscyphus pseudoalbidus* on *Fraxinus excelsior, Plant Pathology,* in press.

Clifford, D.R., Gendle, P., Holgate, M.E., Lulham, M. (1981). Eradication of over-wintering mildew (*Podosphaera leucotricha*) in apple buds by nitrogen-containing surfactants. *Pesticide Science* **12**, 509-514.

Cooke, L.R. (1999). The influence of fungicide sprays on infection of apple cv. Bramley's Seedling by *Nectria galligena*. *European Journal of Plant Pathology*, **105**, 783-790.

Greig, B.J.W. (1976). Biological control *of Fomes annosus* by *Peniophora gigantea. Forest Pathology* **6**, 65-71.

Gross, A., Holdenrieder, O. (2013). On the longevity of *Hymenoscyphus pseudoalbidus* in petioles of *Fraxinus excelsior. Forest Pathology,* in press.

Gross, A., Zaffarino, 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.

Husson, C., Caël, O., Grandjean, J.P., Nageleisen, L.M., Marçais, B. (2012). Occurrence of *Hymenoscyphus pseudoalbidus* on infected ash logs. *Plant Pathology* **61**, 889-895.

James, D., Varga, A., Becker, E., Sumampong, G., Bailey, K., Elliott, M., Masri, S., Shamoun, S.F. (2012). Screening of several disinfectants to assess their efficacy in controlling mycelia growth, sporangia germination, and recovery of viable *Phytophthora ramorum*. *Crop Protection* **42**, 186-192.

Labuschagne, N., Kotzé, J.M. (1996). Control of groundnut blackhull and its causal fungus *Chalara elegans. Plant Pathology* **45**, 540-546.

MacAntSaoir, S.S., Cooke, L.R., McCracken, A.R. (2010). The effects of leaf litter treatments, post-harvest urea and omission of early season fungicide sprays on the overwintering of apple scab on Bramley's Seedling grown in a maritime environment. *Irish Journal of Agricultural and Food Research*, **49**, 55-66.

McDonnell, G., Russell, A. D. (1999). Antiseptics and Disinfectants: Activity, Action, and Resistance. *Clinical Microbiology Reviews* **12**, 147-179.

Schumacher, J., Kehr, R., Leonhard, S. (2010). Mycological and histological investigations of *Fraxinus excelsior* nursery saplings naturally infected by *Chalara fraxinea. Forest Pathology* **40**, 419-429.

Solheim, H., Timmermann, V., Børja, I., Hietala, A.M. (2011). Askeskuddsjuke er på frammarsj. *Skogeieren* **96**, 34-36.

Timmermann, V., Børja, I., Hietala, A.M., Kirisits, T., Solheim, H. (2011). Ash dieback: pathogen spread and diurnal patents of ascospore dispersal, with special emphasis on Norway. *EPPO Bulletin* **41**, 14-20.

Webb, R., Philips, A., Speare, R., Connolly, J., Berger, L. (2012) Controlling wildlife fungal disease spread: *in vitro* efficacy of disinfectants against *Batrachochytrium dendrobatidis* and *Mucor amphibiorum*. *Diseases of Aquatic Organisms* **99**, 119-125.

Worrell, R. (2013). An assessment of the potential impacts of ash dieback in Scotland. Report commissioned by the Forestry Commission Scotland, 52 pp.

Appendix 1. US Risk assessment and comments

NEW PEST ADVISORY GROUP (NPAG), Plant Epidemiology and Risk Analysis Laboratory, Center for Plant Health Science & Technology, USA

Control: There is no information on effective control of *Chalara fraxinea* available. No resistant clones of European ash have been found so far (Lingren, 2008). Chemical control methods have had some success with other *Chalara* spp. (CABI, 2007; Labuschagne and Kotzé, 1996), and may be effective against *C. fraxinea* in seeds. Preventive measures such as sanitation, cultural methods, chemical control, and genetic resistance are important to prevent infection from *Chalara* spp. (Kile, 1993). Sanitation of equipment used near infected trees may reduce spread of the fungus (Kile, 1993; Norwegian Food Safety Authority, 2008). The effectiveness of wood treatments such as heat treatment and methyl bromide against this fungus is uncertain.

Appendix 2. Biocides and disinfectants for possible use in ash dieback control

Product	Active ingredient(s)	Company	Intended use	Used by/for	Source of information
Novagen F.P.	phosphoric acid and iodine	Industrial & Veterinary Hygiene	animal disease disinfection	DARD Vet Service for biosecurity and animal health disinfection	DARD Vet. Service
Total Farm Disinfectant	'broad spectrum iodophors', contains sulphuric acid, phosphoric acid and iodine	Downland Marketing Ltd	animal disease disinfection	DARD Vet Service for biosecurity and animal health disinfection	DARD Vet. Service
Klercide	50-70% ethanol ('denatured ethanol')	Shield Medicare	hard surface disinfection, supplied in sprayer	DARD AfIB, use for disinfection of sampling tools (secateurs etc) including ash dieback surveys	AfIB
Cleankill	alkyl dimethyl benzyl ammonium chloride (benzalkonium chloride), didecyl dimethyl ammonium chloride, chlorhexidine digluconate	Clinimax Ltd, LG Hygiene Ltd.	water-based hand scrub containing 3 bactericides	DARD AFiB use for general biosecurity eg, disinfection of boots, vehicle wheels etc including ash dieback surveys	AfIB
Propellar	isopropanol	Evans Chemical Supplies	spray for disinfection, specifically mentions ash dieback	Forestry Commission for biosecurity	Forestry Commission
Mikrozid AF	ethanol (25%), propanol (75%)	Schülke Products	surface disinfection, spray and wipes	AFBI for disinfecting microflows	AFBI
Jet 5	5% peroxyacetic acid	Certis	disinfection	potato store disinfection	AFBI, SAC, Potato Council
Sorgene 5	peracetic acid, hydrogen peroxide	BASF	disinfection including farm and vehicle disinfection	potato store disinfection	AFBI, SAC, Potato Council
Endoquat	quaternary ammonium compounds	Biohealth Solutions, Endo Enterprises	disinfection: spray, bulk	medical and health disinfection	Fera
Endosan	stabilised hydrogen peroxide	Endo Enterprises	disinfection	"Ecological disinfectant" and biofilm remover	Fera
Envirocair	quaternary ammonium compounds	Biotech International Ltd, Eco Bioguard	disinfection: spray, wipes	medical and health disinfection	Fera
Salvox	hypochlorous acid	Biomimetics Health Industries UK	disinfection: spray, wipes and fogging formulation	medical disinfection	Fera

Appendix 3. Fungicide products with current 'off label' authorisations in Forestry and Amenity Vegetation

Source Liaison database (supplied by CRD)

Amenity Vegetation: Please note this does not automatically permit use in hedgerows

Active(s)	Product	Marketing Company	Crop(s)	Pest(s) / Disease(s) (See Authorisation for details.)
boscalid/pyraclostrobin	Signum	BASF	Amenity vegetation, Interior landscapes	Botrytis, leaf spot (*Septoria*), leaf spot (*Septoria apiicola*), leaf spot (*Botrytis*), powdery mildew, powdery mildew (*oidium*), powdery mildew (*sphaerotheca humuli*), rust (*Puccinia asparagi*), rust (*Puccinia hieracii*), rust (*Uromyces appendiculatus*), rust (*Uromyces fabae*), rust

Forestry:

Active(s)	Product	Marketing Company	Crop(s)
azoxystrobin	e.g. Amistar	Syngenta Crop Protection UK	Outdoor all edible crops (seed crop), Outdoor all non-edible crops (seed crop), Outdoor forest nursery, Protected and outdoor ornamental plant production, Protected and outdoor soft fruit, Protected forest nursery
chlorothalonil	e.g. Bravo 500	Syngenta Crop Protection UK	All edible crops (seed crop), All non-edible crops (seed crop), Outdoor forest nursery, Outdoor ornamental plant production
propiconazole	Bumper 250 EC	Makhteshim-Agan (UK)	Hops, Outdoor forest nursery, Protected forest nursery, Soft fruit
mancozeb	e.g. Dithane 945	Indofil Industries	Forest nursery, Outdoor ornamental plant production
fluoxastrobin and prothioconazole	Fandango	Bayer CropScience Limited	Outdoor forest nursery
mepanipyrim	Frupica Sc	Certis	Forest nursery
cyprodinil	Kayak	Syngenta Crop Protection UK	Outdoor forest nursery
iprodione	Rovral WG	BASF plc	Outdoor forest nursery, Protected and outdoor hops, Protected and outdoor soft fruit, Protected and outdoor top fruit, Protected forest nursery
pyrimethanil	Scala	BASF plc	Forest nursery, Outdoor top fruit
boscalid/pyraclostrobin	Signum	BASF plc	Protected and outdoor forest nursery
trichoderma asperellum, strain T34	T34 BIOCONTROL	Fargro Limited	Forest nursery - container grown crops, Ornamental plant production - container grown crops, Protected forest nursery, Protected ornamental plant production, Protected ornamental plant production - container grown crops
fenhexamid	Teldor	Bayer CropScience	Outdoor hops, Outdoor soft fruit, Protected forest nursery
spiroxamine	Torch Extra	Bayer CropScience	Outdoor forest nursery

Appendix 4. Fungicide products with current on label authorisations for use on apples for apple scab

Source Liaison database (supplied by CRD), shortened to show only one product example is given for each active ingredient

Active(s)	Product	Crop	Target	Method of application	Crop stage	Marketing Company	Comments
boscalid/pyraclostrobin	Bellis	Apples	apple scab	Ground spray	Field application	BASF plc.	Control. Apply as a protectant spray from bud burst and repeat at 10-14 day intervals.
cyprodinil/fludioxonil	Switch	Apples	scab	Ground spray	Field application	Syngenta Crop Protection UK	Reduction when applied as part of spray programme.
dithianon	e.g. Dithianon WG	Apples	scab	Low volume	Field application	BASF plc.	Control. Apply from the beginning of bud burst. Repeat at 7 to 14 day intervals until danger of scab attack ceases. Apply in tank mix for improved control and repeat at 7-10 day intervals.
dithianon/pyraclostrobin	Maccani	Apples	apple scab	Ground spray	Field application	BASF plc.	Control. Use for prevention.
dodine	e.g. Radspor FL	Apples	scab	Ground spray	Field application	e.g. Truchem Ltd.	Control: Apply a maximum of 2.2 litres per hectare.
fenbuconazole	e.g. Indar 5EW	Apples	scab	Ground spray	Field application	e.g. Landseer Ltd	Protection. Apply from bud burst to the onset of petal fall. After petal fall apply in tank mix.
kresoxim-methyl	e.g. Stroby WG	Apples	scab	Ground spray	Field application	e.g. BASF plc.	For optimum results apply from bud burst prior to disease development and repeat at 10-14 day intervals. Where disease pressure is high use the shorter spray interval for optimum disease control.
mancozeb	e.g. Karamate Dry Flo Neotec	Apples	apple scab	Ground spray	Field application	e.g. Landseer Ltd	Control. Apply from bud burst onwards until spore discharge ends. In periods of high pressure apply in tank mix but alternate with other fungicides every 7 days. Repeat application alone/in tank mix cannot be made until 14 days pass, or apply at 10 day intervals in tank mix for optimum control.
myclobutanil	e.g. Systhane 20 EW	Apples	apple scab	Ground spray	Field application	e.g. Landseer Ltd	To control apple scab, apply from bud burst to the onset of petal fall. To improve control of fruit scab after the onset of petal fall, use in tank mix.
pyrimethanil	e.g. Scala	Apples	scab	Ground spray	Field application	e.g. BASF plc.	Control. Apply at start of bud burst and treat at intervals of 10-14 days depending on disease pressure.

Appendix 5. The Food & Environment Research Agency

Shortlist of products for testing against *Chalara fraxinea*

http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/fungicideListForScreening20March2013.pdf

The products listed in the tables below have been shortlisted as candidates for Defra funded field and laboratory testing against *Chalara fraxinea*. They have been selected from more than 50 products suggested by companies that are members of the Crop Protection Association and a further 34 compounds proposed by other companies and individuals. The shortlisting has been carried out by the Fera Plant Pathology Team in consultation with the companies and individuals that have made proposals. Two of the most important criteria used for selecting products were 1) products that have been shown to have activity against *Chalara fraxinea* or other species with a similar lifecycle such as apple scab (*Venturia inaequalis*) 2) products that are already registered as plant protection products in the UK, elsewhere in the EU or are close to achieving registration. The products that have been selected include examples of the fungicide groups which are considered most likely to be effective and, where possible, products with a single active ingredient have been preferred to mixtures for the purpose of the laboratory screen. Additional products are being tested by individual agrochemical companies and further products including mixtures may be tested once the results from this initial screen are available. The most promising products in laboratory screens will be tested as protectant treatments in field trials using ash saplings which will be set up in spring 2013. We are very aware of the possible fungicide resistance risks associated with the pathogen and will be selecting treatments which will minimise the risk of resistance development. Copper oxychloride has been included at the request of stakeholders for a copper based compound to be tested.

Initial products selected for testing for fungicidal activity against *Chalara fraxinea* in laboratory tests*

	Active Ingredient	Product	Fungicide Group	Manufacturer/Contact point
1	Myclobutanil	Systhane 20EW	Triazole	Dow Agrosciences Ltd
2	Cyproconazole	Alto 100 SL	Triazole	Syngenta Crop Protection Ltd
3	Prothioconazole	Proline	Triazole	Bayer CropScience AG
4	Fenbuconazole	Indar 5EW	Triazole	Dow Agrosciences Ltd
5	Flutriafol	Consul	Triazole	Cheminova
6	Azoxystrobin	Amistar	Strobilurin	Syngenta Crop Protection Ltd
7	Fluxapyroxad	Imtrex	SDHI#	BASF plc
8	Bixafen/prothioconazole	Aviator 235 XPro	SDHI/triazole	Bayer CropScience AG
9	Boscalid/pyraclostrobin	Signum	SDHI/strobilurin	BASF plc

10	Mancozeb	Cleancrop Mancozeb	Dithiocarbamate	InterFarm (UK) Ltd
11	Pyrimethanil	Scala	Anilinopyrimidine	BASF plc
12	Dithianon	Dithianon WG	Quinone	BASF plc
13	Garlic extract (allicin)	n/a	Organosulfur	To be confirmed
13	Copper oxychloride	Cuprokylt FL	Inorganic copper	Universal Crop Protection

* - further products may be tested based on results from the initial screening

- succinate dehydrogenase inhibitor

Additional products selected for testing for activity against *Chalara fraxinea*

	Active Ingredient	Comments
1	Potassium phosphite	To be tested as a stimulant of host resistance in field trials using ash saplings
2	Urea	To be tested as a foliar spray applied to ash saplings and treatment of leaf litter for suppression of development of apothecia and promotion of leaf decomposition

20 March 2013