Hymenoscyphus fraxineus

Synonyms:
Chalara fraxinea Kowalski (anamorph), *Hymenoscyphus pseudoalbidus* (teleomorph).

Common Name(s)
Ash dieback, ash decline

Type of Pest
Fungal pathogen

Taxonomic Position
Class: Leotiomycetes**, Order:** Helotiales, **Family:** Helotiaceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2010-2016

Figure 1. Mature *Fraxinus excelsior* showing extensive shoot, twig, and branch dieback. Epicormic shoot formation is also present. Photo credit: Andrin Gross.

Background
An extensive dieback of ash (Fig. 1) was observed from 1996 to 2006 in Lithuania and Poland. Trees were dying in all age classes, irrespective of site conditions and regeneration conditions. A fungus, described as a new species *Chalara fraxinea*, was isolated from shoots and some roots (Kowalski, 2006). The fungal pathogen varied from other species of *Chalara* by its small, short cylindrical conidia extruded in chains or in slimy droplets, morphological features of the phialophores, and by colony characteristics.

Initial taxonomic studies concerning *Chalara fraxinea* established that its perfect state was the ascomycete *Hymenoscyphus albidus* (Gillet) W. Phillips, a fungus that has been known from Europe since 1851. Kowalski and Holdenrieder (2009b) provide a description and photographs of the teleomorphic state, *Hymenoscyphus albidus*.

A molecular taxonomic study of *Hymenoscyphus albidus* indicated that there was significant evidence for the existence of two morphologically very similar taxa, *H. albidus*, and a new species, *Hymenoscyphus pseudoalbidus* (Queloz et al., 2010). Furthermore, studies suggested that *H. albidus* was likely a non-pathogenic species, whereas *H. pseudoalbidus* was the virulent species responsible for the current ash dieback epidemic in Europe (Queloz et al., 2010). A survey in Denmark showed that expansion of *H. pseudoalbidus* has resulted in *H. albidus* becoming a rare species, possibly because the two fungi occupy the same niche (Pautasso et al., 2013).

Figure 2. *Hymenoscyphus fraxineus* teleomorph (*H. pseudoalbidus*) on an ash petiole (left). Pseudothecia on an infected petiole (right). Photo credit: Ottmar Holdenrieder.

The ash dieback pathogen has been on the CAPS prioritized pest list for several years. It was initially listed as *Chalara fraxinea* (the anamorphic/ asexual stage). In 2014, we added *Hymenoscyphus pseudoalbidus* (the teleomorphic/sexual stage) to the name. As a result of a large international effort to assign one name to each fungus, the suggested name has changed to *Hymenoscyphus fraxineus* (Baral et al., 2014).

Pest Description

From Gross et al., (2014):

Teleomorph:

Hymenoscyphus fraxineus is a discomycete that forms numerous white-stalked apothecia (mostly up to 3 mm in diameter, rarely 8 mm) on the leaf litter of the previous year (Fig. 2). The hymenium is composed of cylindrical paraphyses (1.8–2.4 µm thick, enlarged to 3 µm at the apex) and cylindrical-clavate asci (80–107 × 8–10 µm), showing a positive iodine reaction and forming hyaline single-celled ascospores (13–21 × 3.5–5.0 µm). During germination, the ascospores become melanized and single septate in most cases, and appressoria and/or germ tubes are formed. The substrate colonized by *H. fraxineus* (leaf debris in the litter, occasionally also small stems) becomes blackened by a conspicuous pseudosclerotial layer on which the apothecia develop during the summer.

Figure 3. Colony morphology of *C. fraxinea* (anamorph) isolated from symptomatic tissue on malt extract agar. Photo from Kowalski and Holdenrieder (2009a).

From Kowalski (2006):

Anamorph:

Colony morphology: Colony on malt extract agar (MEA) effuse, cottony, dull white to fulvous brown (dull reddish-yellow/orange or browny-yellow) (Fig. 3), with some patches becoming gray to dark gray, 9-28 mm diameter after 21 days at 20°C (68°F) in the dark. Vegetative hyphae subhyaline to olivaceous brown, 1.2-3.0 µm wide with rare swellings up to 4.2 µm; thin-walled, smooth, septate with septa 5-21 µm apart. Chlamydospores

absent. Some cultures older than 2 weeks with patchy or linear pseudoparenchymatous stroma composed of cells with thickened, dark-brown walls. No growth was observed on MEA amended with 0.1 g cycloheximide per liter.

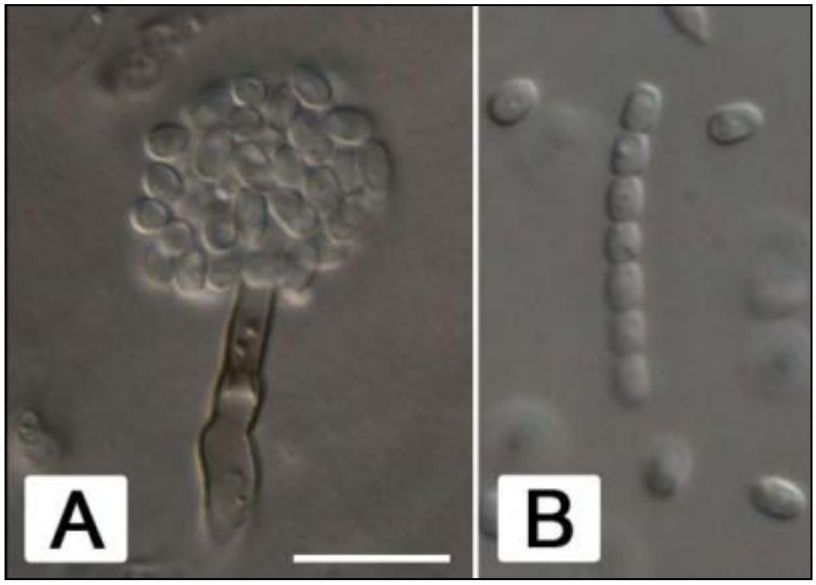

Figure 4. Conidia of *C. fraxinea* anamorph. A) In a slimy droplet and B) in a chain. Bar = 10 µm. Photo courtesy of Ogris et al. (2009).

Phialophores: Arising directly on the superficial or slightly immersed hyphae on pseudoparenchymatous stroma or solitary and scattered, often reduced to phialides, or cylindrical to obclavate up to three septate in the basal part, olivaceous brown, erect, straight or slightly bent, smooth-walled, unconstructed at the septa, mainly 24-37 µm long, terminating in a phialide. In few-week-old colonies, phialophores up to 96 µm long and 3.0-4.2 µm wide at base, simple or with one to five branches. Phialides occur also terminally on undifferentiated hyphae.

Phialides: Subcylindrical to obclavate, occasionally lageniform, 16-24 µm long. Venter short-cylindrical to ellipsoidal, 11-15 x 4-5 µm. Collarette cylindrical, 5-7/9/ x2.2-2.7; ratio of mean length of collarette to venter = 0.6:1. Transition from venter to collarette gradually, occasionally abrupt.

Conidia (phialoconida): Extruded in short chains or more frequently in slimy droplets (Fig. 4); short-cylindrical, ends rounded or blunt, sometimes with a truncate base bearing small marginal frills, unicellular, hyaline to subhyaline, filled with one or two oil droplets, smooth-walled, 3.2-4.0 x 2.0-2.5 µm; mean conidium length/width ratio 1.4:1. The first forming conidium is shortly clavate 6-7 x 2.2-2.5 µm.

Biology and Ecology

Hymenoscyphus fraxineus is heterothallic and reproduces sexually on ash petioles in the litter once a year. Ascospores are wind dispersed and infect ash leaves during the summer. The asexual spores presumably serve as spermatia (Gross et al., 2014). The entire life cycle of *H. fraxineus* is completed on *Fraxinus* leaves. Apothecia are produced during the summer on leaf debris of the previous year. Most apothecia are formed on petioles but, occasionally, apothecia are also found on small stems lying on the ground. The main sporulation period is from June to early September, but, under favorable conditions, sporulation can start earlier and last until October. The wind-dispersed ascospores adhere to the leaf surface via a secreted mucilage, which is more

or less hyaline around the spore and conspicuously melanized around appressoria formed on cellophane on ash leaf agar. Ascospores penetrate the ash leaf cuticle via appressoria. In moist chambers, initial lesions on the leaves of ash seedlings occurred 2 weeks after inoculation (Cleary et al., 2013b). After leaf fall in the autumn, the fungus starts to produce a characteristic black pseudosclerotial plate on the petioles, occasionally also on leaflet veins and small stems. The pseudosclerotial plate is formed a few cell layers below the substrate surface and the peripheral host tissues are degraded by other organisms until the spring, so that the melanized wall of the pseudosclerotium becomes exposed. In the following vegetation period, during the second or up to the fifth year after leaf fall, new apothecia develop on the pseudosclerotia and the cycle is closed (Gross et al., 2014; Kirisits, 2015).

Figure 5. Small necrotic lesions on shoots of young *F. excelsior* prior to budburst. Photo from Kirisits et al. (2008).

Hymenoscyphus fraxineus may be able to disperse aerially, but is more likely to move in soil, water, plants for planting, or wood (NPAG, 2009; EPPO, 2010a). The biggest danger for dispersal is through infected petioles. Gross at al. (2012) found up to eight different genotypes on a single ash petiole. Therefore, a small fragment of a petiole might be enough to start a new epidemic somewhere else. These fragments can be moved by soil, water or on infected plants. Another dispersal danger comes from spread through infected wood (Husson et al. 2011), on which the imperfect state can be formed and in rare cases also apothecia can be produced. Last but not least, infected seeds also pose a risk for dispersal of this pathogen.

A study by Cleary et al. (2013a) found that *H. fraxineus* DNA remained in *Fraxinus excelsior* (European ash) seed for up to two years post-harvest, suggesting that *H. fraxineus* may be transmitted by seed. However, the viability of fungal propagules and their ability to incite disease in developing embryos was not thoroughly investigated in that study. A follow up study examined the vertical transmission of *H. fraxineus* from seed to germinating seedling. This essentially did not happen. Therefore, seeds pose a very low risk as a potential infection pathway for the disease (Cleary, 2015, personal communication).

Initially, the exact role of *H. fraxineus* in ash dieback was not clear, as it was commonly found in ash trees that are also colonized by other potentially pathogenic fungi including species of: *Armillaria*, *Cytospora*, *Diplodia*, *Fusarium*, and *Phomopsis* (Bakys et al., 2009b; Kowalski and Holdenrieder, 2009a). Vasaitis and Lygis (2008) postulated that 1) *H. fraxineus* may be a natural component in the microfungi on ash trees and its pathogenicity may have been triggered by environmental factors, or 2) *H. fraxineus* may be a new invasive species gradually spreading over new areas along with changing climate.

Figure 6. Canker caused by *H. fraxineus* on *F. excelsior* (left) and canker with bark removed (right). Photos courtesy of H. Solheim. Norwegian Forest and Landscape Institute, Aas, Norway. www.eppo.org.

Kowalski and Holdenrieder (2009a) demonstrated the pathogenicity of *H. fraxineus* by field inoculation of young *Fraxinus excelsior* trees. Bakys et al. (2009a) were able to provide further experimental evidence for the pathogenicity of *H. fraxineus* to *F. excelsior*. In total the authors showed the presence of 25 different fungal taxa on European ash via sequence results. *H. fraxineus* was isolated from 93% of stem cankers, 91% of necrotic leaf stalks, 27-28% of bark wounds, and 30% of visually healthy leaf stalks. In another study, Bakys et al. (2009b) were able to isolate 56 different fungi from symptomatic *F. excelsior* shoots from central Sweden; the most pathogenic species in inoculation studies was *H. fraxineus*. Most European scientists have now concluded that *H. fraxineus* is the primary causal agent of ash dieback (Kirisits et al., 2008; Kowalski and Holdenrieder 2009a).

In a preliminary analysis of genetic variation, a considerable amount of genetic variation was detected among 20 Finnish, one Latvian, and 11 Estonian isolates of *H. fraxineus* analyzed by random amplified microsatellite (RAMS) markers (Rytkönen et al., 2010). The fingerprint patterns produced by three RAMS primers revealed five variable and eight non-variable markers that were clear, reproducible, and easily scored. Polymorphisms of the five variable RAMS markers separated the 32 isolates into 14 haplotypes. Because a newly introduced species is characterized by low genetic variation, these results exclude the hypothesis that the dieback epidemic in these countries was caused by a single strain of *H. fraxineus* (Rytkönen et al., 2010).

Figure 7. Leaf vein necrosis (top), and leaf spotting and wilting (bottom) in infected ash hosts. Vein necrosis tends to extend towards the petiole. Photo credit: Ottmar Holdenrieder, Andrin Gross.

Symptoms/Signs

Ash dieback has been observed not only on forest trees but also in urban areas (parks and gardens) and nurseries. Although ash trees of all ages are affected, mortality is more common amongst saplings. In areas with some of the longest dieback history such as Lithuania (15+ years of living with the disease), mature ash stands are dying as well. The cumulative effect of annual infections draws substantial resources from the host, reducing growth and predisposing trees to secondary infection by other agents (Cleary, 2015, personal communication).

Initial symptoms include leaf spotting, vein necrosis towards the petiole, necrosis on petioles and partial or complete leaf wilting (Fig. 7). Later on, small necrotic spots (without exudate) appear on the stems and branches (Fig. 5, 6). These necrotic lesions then enlarge and might the girdle shoot resulting in wilting and premature shedding of leaves, dieback of branches, shoots, and twigs, and particularly in the death of the top

Figure 8. Collar necrosis in infected ash. Courtesy of Anne Chandelier, CRAW, Belgium.

of the crown. A brownish to grayish discoloration of the wood that often extends in a longitudinal direction beyond necrotic areas in the bark has been observed in Austria (Halmschlager and Kirisits, 2008). Affected trees show prolific formation of epicormic shoots (shoots that grow from epicormic buds found under the bark of trunks, stems or branches) (Fig. 1). Epicormic buds lie dormant beneath the bark, their growth suppressed by hormones from active shoots higher up the plant. Under certain conditions, they develop into active shoots, such as when damage occurs to higher parts of the plant or light levels are increased following removal of nearby plants.

In addition to the aforementioned symptoms caused by *H. fraxineus*, collar necrosis (Fig. 8) near the tree base has shown to be common in infected host strands and is likely to accelerate the declining process of ash dramatically (Husson et al., 2012; Enderle et al., 2013). Fungi from the species *Armillaria* are also commonly found on collar rot of ash hosts along with *H. fraxineus* and may act as a secondary agent of mortality. It has not yet been excluded that *Armillaria* spp. causes the initial collar necrosis in ash hosts and *H. fraxineus* infects the tree base as a secondary pathogen. Either way, infection at the tree base by *H. fraxineus* is a major contributing factor to host mortality caused by ash dieback (Enderle et al., 2013).

The disease is often chronic but can be lethal. According to Skovsgaard et al. (2010), statistically-based analysis showed that dieback is the primary symptom of disease associated with *H. fraxineus* infection, and the symptoms attributed to dieback were strongly associated with canker in the crown.

Black ash tree symptoms include wilting of leaves, dieback and necrotic lesions of shoots and twigs, and death of canopy. Green ash trees were moderately affected (with symptoms similar to black ash, but with less evidence of dead shoots within the canopy). White and Manchurian ash trees were the least affected with symptoms including wilting of leaves, but only minor shoot and twig dieback and bark necrosis (Drenkhan and Hanso, 2010). However, these observations were made on only few trees and there is still very little information about the susceptibility of ash species other than *F. excelsior*.

Pest Importance

In Lithuania by 2002, over 30,000 ha of *F. excelsior* stands were affected by ash dieback, resulting in mortality of approximately 60% of all ash stands state-wide, while in certain parts of the country only about 20% of *F. excelsior* remained visually healthy (Visaitis and Lygis, 2008; Bakys et al., 2009b). This extensive dieback of ash is spreading throughout Europe at an alarming rate and is of concern for ash forests and ash used in nurseries and landscape environments. The disease has not just spread over long distances, but has rapidly caused high levels of ash tree mortality in all age classes. For example, in Denmark ash dieback was first observed in 2003, became common by 2005 (particularly on young stands), by 2008 was reported as extensive on about one third of all monitored ash trees and by 2009 was affecting ash trees of all ages in all parts of the country (Pautasso et al., 2013).

Fraxinus spp. are present in all 48 states in the continental United States (BONAP, 2015). They are common in both forests and in urban landscaping. Ash trees in North America already face a dire threat from the emerald ash borer (*Agrilus planipennis*), and the establishment of *H. fraxineus* would likely exacerbate this threat.

Known Hosts

Hymenoscyphus fraxineus is known to infect *Fraxinus* spp. (ash). There is a variation in susceptibility to this fungus depending on the type of ash (Table 1) (Gross et al., 2014).

Host	Comon name	Susceptibility
Fraxinus angustifolia	Narrow-leafed ash	high
Fraxinus excelsior	European, common ash	high
Fraxinus nigra	Black ash	high
Fraxinus pennsylvanica	Green ash	Moderate
Fraxinus americana	White ash	low
Fraxinus mandschurica	Manchurian ash	low

Table 1. Ash hosts of *H. fraxineus* and their susceptibility to the pathogen.

Cleary et al. (2015) report that North American ash species (black, green, and white) are all susceptible to *H. fraxineus;* while Asian ash species are not susceptible. In addition, they report that *H. fraxineus* has been found on asymptomatic *F. mandchurica* (Manchurian ash) and may play a role as an endophyte on this species.

Fraxinus ornus (flowering ash), is moderately susceptible to *H. fraxineus* under experimental conditions (Kirisits et al., 2009; Gross et al., 2014), and was recently shown to be infected in a forest under massive natural infection pressure (Krisits and Schwanda, 2015). However, natural infection appears to be uncommon in this host.

Known Vectors (or associated insects)

H. fraxineus does not have a known vector or associated organisms. Other *Chalara* spp., the anamorph of *H. fraxineus*, have known insect vectors, there are no reports of an insect vector for *H. fraxineus* at this time.

Known Distribution

Asia: China, Japan, South Korea, Russia Far-East. **Europe:** Austria, Belarus, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Lithuania, Luxembourg, Norway, Poland, Romania, Russia, Slovakia, Slovenia, Sweden, Switzerland, Ukraine, and United Kingdom (Schumacher et al., 2007; Kowalski and Holdenrieder, 2009a; Ogris et al., 2009; Szabo, 2009; Talgo et al., 2009; EPPO, 2010a,b; Drenkhan and Hanso, 2010; Ogris et al., 2010; Skovsgaard et al., 2010; Chandelier et al., 2011; Davudenko et al., 2013; Garnier-Delcourt et al., 2013; Pautasso et al., 2013; Zheng and Zhuang, 2013; Han et al., 2014; McKinney et al., 2014; Cleary et al., 2015).

Pathway

Currently, the import of *Fraxinus* spp. propagules (except seed) is prohibited from all countries except parts of Canada (USDA, 2015). Canada is not known to have *H. fraxineus.* The import of *Fraxinus* spp. seed material, however, is currently allowed without restriction (USDA, 2015). From 2003-2007, the United States imported an average of 22 metric tons of *Fraxinus* spp. seed from Europe per year (NPAG, 2009). The United States imports ash wood from Europe, although such imports have been steadily declining (NPAG, 2009). A recent study suggests that *H. fraxineus* may remain viable in ash seed for two years (Cleary et al., 2013). Thus, it may be possible that this fungus can be introduced into a new locality by means of untreated infected seeds, if those seeds were collected from areas within the current zone of infestation that includes the larger part of continental Europe. However, a follow up study by Cleary (2015, personal communication), shows that the risk of vertical transmission from infected seed to germinating seedlings is very low.

Since 2005, there have been interceptions of *Fraxinus* spp. plant material intended for propagation from the following countries which are known to have *H. fraxineus*: China (9), Japan (3), South Korea (2), Hungary (1), and Romania (1) (AQAS, 2015). The majority of these interceptions (10) were seed.

Potential Distribution within the United States

At this time the full host range of *H. fraxineus* is not known. Therefore, it is not known if the 16 native species of ash within the United States (NRCS, 2010) are susceptible to this pathogen. Even though host/non-host experiments have not been performed for *H.*

fraxineus, it may infest other species of *Fraxinus*. *Fraxinus angustifolia* is not known to occur in the United States. *Fraxinus excelsior*, the primary reported host, has a limited distribution in the United States, found only in limited counties in Connecticut, Kentucky, Massachusetts, New York, and Ohio, as well as New Brunswick, Nova Scotia, Ontario, and Quebec, Canada (NPAG, 2009; NRCS, 2010; BONAP, 2015). Based on the presence of all *Fraxinus* species in the United States, the Eastern half of the country has the greatest potential risk for establishment of this pathogen (BONAP, 2015).

Survey

Approved Method for Pest Surveillance (AMPS)*: Visual survey is the approved survey method for *H. fraxineus*. For visual survey, collect symptomatic plant material. Symptoms include: Leaf vein necrosis, epicormic shoot formation, shoot, twig, and branch dieback, wilting, leaf and bark lesions, and gray to brown discoloration of the wood.

A heavily melonized pseudothecial layer may form on petioles (Fig. 2) (Gross, 2015, personal communication). Visual detection of this layer is a very good way to detect the pathogen in the earliest stages of the epidemic when symptoms on leaves and branches are still rare. On the leaves, the veinal necrosis is a characteristic symptom.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at https://caps.ceris.purdue.edu/node/223.

Literature-Based Methods:

Generally trees showing symptoms of crown decline are sampled (Rytkönen et al., 2010; Bakys et al., 2009b). One to four symptomatic (*i.e*., having necrotic lesion) twigs or branches were collected from each symptomatic trees (Rytkönen et al., 2010). In each stand in Sweden, branches with various severity of dieback were cut from three to five trees, individually packaged into plastic bags for transport (Bakys et al., 2009b).

Key Diagnostics/Identification

Approved Method for Pest Surveillance (AMPS)*: Confirmation of *H. fraxineus* requires a morphological identification. Most frequently, the pathogen is isolated on malt extract agar (supplemented with streptomycin sulfate) from the leading edge of discolored wood and necrotic leaf petioles, but rarely from necrotic bark (Kowalski, 2006).

Kowalski (2006) notes that *H. fraxineus* differs from other species of *Chalara* by its small, short, cylindrical phialoconidia extruded in chains or in slimy droplets, morphological features of the phialophores, and by colony characteristics.

Molecular methods are being validated by the CPHST Beltsville laboratory. The approved methods will be updated after the validation is completed.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at https://caps.ceris.purdue.edu/node/223.

Literature-Based Methods:

Morphological: The easiest way to isolate the fungus is to isolate it from pseudosclerotial leaf petioles (Gross and Holdenreider, 2013; Gross et al., 2014). The pathogen can also be isolated from main shoots and twigs by surface sterilizing the material, removal of surface bark, and plating pieces of shoots 5 x 2 x 2 mm on Petri plates containing 2% malt extract agar supplemented with 100 mg/L of streptomycin sulphate. Cultures are incubated at room temperature in the dark (Kowalski, 2006). Because of its slow growth rate on agar medium and the frequent presence of fast-growing saprotrophic fungi with the host tissue, classical isolation techniques are time-consuming and sometimes inefficient (Ioos et al., 2009). Bakys et al. (2009b) used Hagem agar medium to isolate fungi from diseased ash. Talgo et al. (2009) used potato dextrose agar and water agar for pathogen isolation.

Molecular: Bengtsson et al. (2012) reviews species-specific identification of *H. fraxineus* using ITS (internal transcribed spacer) markers of ribosomal genes. Chandelier et al. (2009) developed a real-time PCR for the detection of *H. fraxineus* from woody tissues. A procedure for DNA extraction from woody tissues using an electric drill yielded DNA of appropriate quality for DNA extraction. PCR primers and Taqman probes were developed based on the internal transcribed spacer (ITS) region of a multi-copy gene rDNA. The primers amplified an 81 bp fragment from *H. fraxineus*. The limit of detection was 5 pg of genomic DNA per PCR. This method has proven to specifically amplify *H. fraxineus* successfully.

Ioos et al. (2009) developed a real-time PCR for the detection of *H. fraxineus in planta*. Species-specific polymorphisms observed within the ITS region were used to design a primer pair and a dual-labeled probe for use in a real-time PCR assay for the detection of *H. fraxineus. In silico* and *in vitro* assessments showed that the PCR could detect as little as 20 fg of *H. fraxineus* DNA. This method, however, cannot differentiate between *H. fraxineus* and *H. albidus*. Husson et al. (2011) have improved the method.

Johansson et al. (2010) developed a set of species-specific PCR primers, based on ITS sequences, to detect *H. fraxineus* in plant (*Fraxinus excelsior*) tissue. This method also cannot differentiate between *H. fraxineus* and *H. albidus*. McKinney at el. (2012) have improved the method.

Easily confused sister species of *H. fraxineus*

Besides *H. albidus*, four other known near-cryptic sister species of *H. fraxineus* were recently identified. *Hymenoscyphus albidoides* was described from leaves of *Picrasma quassioides* from China (Zheng and Zhuang 2013). *Hymenoscyphus linearis* was discovered on rotten leaves of *Fraxinus platypoda* (synonym *F. spaethiana*) from Japan (Gross, 2015, personal communication). Finally, *Hymenoscyphus koreanus* and *Hymenoscyphus occultus* were found on rotten *Fraxinus chinensis* subsp. *rhynchophylla* and *Fraxinus mandshurica* leaves in South Korea (Gross and Han 2015). The pathogenicity of these species is mostly unknown (except for *H. linearis*) as well as

their potential to form inter-species hybrids. Therefore, transfer of these species out of their native range must be avoided.

Easily Confused Species

Symptoms of ash dieback could be confused with those caused by emerald ash borer, *Agrilus planipennis* (NPAG, 2009). In addition to exit holes on the trunks and branches, emerald ash borer infestation causes yellowing and thinning of the foliage, dieback of branches, epicormic shoots, and eventually death of the trees (CABI, 2007).

H. fraxineus can also be confused morphologically with *Chalara* species, especially those species with short phialides (*C. austriaca*, *C. microspora*, *C. sessilis*, and *C. fusidiodes*) (Kowalski, 2006). All of these species differ from *H. fraxineus* by the form of the conidia. *Cryptendoxyla hypophloia* also produces short but hyaline phialides, whereas phialides of *H. fraxineus* are pigmented (Kowalski, 2006).

The collar necrosis symptoms caused by *H. fraxineus* resemble collar rot caused by *Phytophthora* spp. in other tree hosts. However, studies have shown that *Phytophthora* spp. are not associated with ash dieback (Enderle et al., 2013).

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Update history:

November, 2014: Added China and Luxembourg to distribution list. Changed name of this pathogen from *Hymenoscyphus pseudoalbidus* (*Chalara fraxinea*) to *Hymenoscyphus fraxineus*.

April, 2015: Complete update of entire datasheet to include current information. Added new photographs. Revised based on review comments from Andrin Gross and Michelle Cleary.

May, 2015: Updated symptoms/signs section to include collar necrosis information and photos. Incorporated comments from Dr. Chandelier.

November, 2016: Added *F. ornus* to natural host list.

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