Ash dieback—Chalara fraxinea

Ash dieback disease was first observed in North and Central Europe in the 1990s (Bakys et al., 2009a; Kowalski and Holdenrieder, 2009b) and is now known throughout Europe. Due to the severity of ash dieback, the conidial fungus Chalara fraxinea has been on the EPPO Alert list since 2007. Although its sexual form, the ascomycete Hymenoscyphus albidus, was previously widely observed as a saprophyte, the fungus now appears to be spreading as a pathogen of Fraxinus excelsior in Europe. It is not known whether a change in the fungus or a changing environment caused the emergence of this "new" disease (NAPPO, 2009). The means of spread of the conidia and/or ascospores has not been established, but infected nursery saplings may carry the fungus to new areas. The natural range of known hosts extends to North Africa, Russia and southwest Asia (USDA-ARS, 2009), but the susceptibility of the other species of ash in temperate zones is not known.

Chalara fraxinea T. Kowalski 2006

Colonies on malt extract agar (MEA) cottony, white, orange-brown or fulvous brown, reverse brownish, grey sectors in areas associated with sporulation. Growth slow, about 1 mm per day at 20° C. Pseudoparenchymatous stromata formed occasionally after prolonged incubation.

Chalaria fraxinea: Hyphae 1.2-3.0 µm broad, subhyaline to olive-brown. Phialophores solitary and scattered, septate, branched or unbranched, olive-brown. Phialides subcylindrical to obclavate, 16-24 µm, olive-brown; venter cylindrical to ellipsoid, 11-15 x 4-5 µm; collarettes cylindrical, 5-7 x 2.2-2.7 µm. Conidia short-cylindrical, hyaline to subhyaline, aseptate, smooth-walled, 2.0-4.0 x 2.0-2.5 µm, ends blunt or rounded, base sometimes truncate, occasionally bearing small marginal frill, in short chains or more often in droplets. First-formed conidia are longer.

Hymenoscyphus albidus: Apothecia scattered, superficial, white to cream, becoming cinnamon brown with age and drying, arising from blackened areas of fallen petioles or dead shoots. Disk flat, 1.5-3.0 mm diam, stipe 0.4-2.0 x 0.2-0.5 mm, enlarged or narrow at base, basal region frequently black. Paraphyses cylindrical, 1.8-2.4 µm thick, enlarged to 3 µm at apex, septate, hyaline, slightly yellowish. Asci cylindric-clavate, stipitate, 80-107 x 6-12 µm, eight-spored. Ascospores irregularly biseriate, fusiform-elliptical, broadly rounded above, narrow below, straight or slightly curved, 13-17 (-21) x 3.5-5 µm, hyaline and aseptate within ascus, becoming 1(-2)-septate and brownish on MEA.

For additional details, see White, 1944; Kowalski, 2006; Halmschlagerand Kirisits, 2008; Kirisits et al, 2009; Kowalski and Holdenrieder, 2009b.

Distribution: Europe, see http://www.pestalert.org/viewNewsAlert.cfm?naid=69. Also, possibly Asia (Japan).

Host: Fraxinus excelsior (Oleaceae), perhaps on other species in the genus.

The anamorphic (asexual) state of the causal agent of ash dieback was described by Kowalski (2006) as Chalara fraxinea based on the structure of its phialides, which have a wide basal venter and a long collarette enclosing a deep-seated site of conidial formation. Later, the apothecial teleomorph (sexual state) was found through morphological and molecular comparisons to be a previously identified species of Hymenoscyphus, H. albidus (Kowalski and Holdenrieder, 2009b). That discomycete genus includes a number of saprophytes on stems, leaf litter, bark and wood (Lizon, 1992), among which H. albidus is not well-known (Kowalski and Holdenrieder, 2009b). It was, for example, not included in an examination of the genus by Baral et al. (2006). The genus determined for the anamorph, then, is not a natural grouping as currently described; Chalara-like anamorphs do occur among discomycetes, but are not common, and are more typically found in perithecial fungi (Nag Raj and Kendrick, 1993; McKenzie et al., 2002). Several other generic names have been used for the teleomorph of this species (Farr and Rossman, 2009). Dennis (1956) synonymized this name as Helotium robergei, but Helotium is determined to be a generic name in the Basidiomycetes (Lizon, 1992). Korf (1982) transferred Hymenoscyphus albidus to Lambertella.

The relationship of H. albidus-C. fraxinea to other species in the genus has not been determined using sequence data (Baral et al, 2006; Zhuang and Liu, 2007). Hymenoscyphus albidus is similar morphologically to H. caudatus (Kowalski and Holdenrieder, 2009b) and Chandelier et al. (2009) found high sequence homology between the two species. Although the apothecia of both of these species develop as saprophytes (Breitenbach and Kranzlin, 1984), some fungi in related genera, such as Crumenulopsis soraria and Cenangium ferrugineum, are parasites on conifers (Sinclair and Lyon, 2005).
Ash dieback disease was first observed in North and Central Europe in the 1990s (Bakys et al., 2009a; Kowalski and Holdenrieder, 2009b). *Chalara fraxinea* was identified as the primary cause in Poland by Kowalski (2006), and was subsequently found in Germany (Schumacher et al., 2007), Sweden (Thomsen et al., 2007), Norway (Jankovský and Holdenrieder, 2009), Denmark (EPPO, 2009a), the Czech Republic (Jankovský and Holdenrieder, 2009), Austria (Harmschlager and Kirisits, 2008) and Hungary (Kirisits et al., 2009; Szabo, 2009). The most recent reports come from south of the Alps in Slovenia and Italy (Ogris et al., 2009, 2010). Based on the teleomorph identification, the fungus also occurs in the United Kingdom (Dennis, 1956; Cannon et al., 1985), Switzerland (Breitenbach and Kranzlin, 1984) and France (White, 1944). The teleomorph was already known to be widespread in the Czech Republic (Jankovský and Holdenrieder, 2009) and elsewhere in Europe (Kowalski and Holdenrieder, 2009b), although White (1944) questioned the accuracy of some of the earlier records. Based on symptoms observed in common ash, *Chalara fraxinea* is also suspected to occur in Estonia, Latvia, and Switzerland (EPPO, 2008).

If the fungus is in fact the same as that identified as *Lambertella albida* on *Fraxinus mandshurica* (Kofr,1982; Kobayashi, 2007), it is also present in Japan and possibly other East Asian areas where that tree species is native (USDA-ARS, 2009). If the fungus identified as *Helotium robergei* occurring on *Aesculus indica*, a host in a different plant family, is the same, then the species occurs in India (Sarbhoy et al., 1971). Additional information is needed regarding the host range and distribution of the teleomorph (NAPPO, 2009).

**RISK OF INTRODUCTION**

The fungus could certainly be distributed from forest nurseries on infected saplings (Kirisits et al., 2009). Windblown ascospores could be dispersed locally, as occurs with the related pathogen *Crumenulopsis soraria* (Hayes, 1980). International concern with the invasive emerald ash borer beetle (USDA/APHIS, 2009; CFIA, 2009; EPPO, 2009) should enhance restrictions on and inspection of any ash logs or lumber that might carry the fungus over great distances.

**SIMILARITIES TO OTHER SPECIES/CONDITIONS**

*Chalara fraxinea* can be distinguished from other *Chalara* species (Nag Raj and Kendrick, 1975; McKenzie et al., 2002) by its small, short cylindrical, aseptate conidia (Kowalski, 2006). The teleomorph is differentiated from other *Hymenoscyphus* species by its occurrence on *Fraxinus* and the dark superficial layer produced on the substrate at the base of the apothecium (Kowalski and Holdenrieder, 2009b).

Unlike most other dieback and canker-causing pathogens known to affect *Fraxinus* (Sinclair and Lyon, 2005), this fungus does not sporulate in pycnidia or perithecia and produces no obvious stromata in or on infected stems or branches. Complicating the diagnosis, some of those other fungi, including *Fusarium* species and *Botryosphaeria stevensii*, may be isolated from necrotic bark lesions caused by *C. fraxinea* (Bakys et al., 2009a, b; Kowalski and Holdenrieder, 2009a; Schumacher et al., 2009).

Symptoms of ash dieback are similar to those caused by the emerald ash borer (NAPPO, 2009), but the beetle is known to attack a wider range of *Fraxinus* species, and the larvae create S-shaped galleries in the sapwood, while emerging adults leave characteristic holes in the bark (APHIS, 2009; CFIA, 2009).

**DETECTION AND INSPECTION METHODS**

Trees can be observed for dieback symptoms, but these may be confused with those caused by other fungi or by insects, and *C. fraxinea* can be present in asymptomatic leaves (Bakys et al., 2009a, b). The other pathogens may be excluded from the diagnosis on the basis of the absence of their characteristic fruiting structures. The *Chalara* anamorph, however, has seldom been observed sporulating in natural lesions (Kowalski and Holdenrieder, 2009a). The apothecia are produced on detached petioles in the leaf litter (Kowalski and Holdenrieder, 2009b), but may also occur on dead shoots (Kowalski and Holdenrieder, 2009a).

**DIAGNOSTIC METHOD**

This fungus cannot be reliably isolated in culture (Lygis et al., 2005; Bakys et al., 2009a; Kowalski and Holdenrieder, 2009a) and is slow-growing (Kowalski and Holdenrieder, 2009b; Schumacher et al., 2009). Other fungi may overgrow it in culture or invade necrotic bark tissues in its cankers (Bakys et al., 2009b).

Three PCR techniques for detection of the fungus in infected plant tissue have been published, each using primers for sequences in the ITS region of rDNA to amplify DNA specific to *C. fraxinea* (Chandelier et al., 2009; Ioos et al., 2009; Johansson et al., 2009). The protocol of Johansson et al. (2009) uses the sequence of an intron in the region unique to this species within the genus *Hymenoscyphus*, while those of Chandelier et al. (2009) and Ioos et al. (2009) were tested against a number of *Chalara* species and other fungi which may be present in or on the lesions. The sequences of ITS regions of rDNA for the teleomorph and the anamorph are available in GenBank for comparison (NCBI, 2009).

**NOTES ON CROPS/OTHER PLANTS AFFECTED**
Only two *Fraxinus* species, *F. excelsior* L. (Kowalski, 2006) and *F. angustifolia* Vahl (Kirisits et al., 2009) are definitely known to be susceptible to the pathogen. Other species in the same section of the genus (USDA-ARS, 2009) include *F. mandshurica* Rupr., from which the sexual state of the fungus has been reported in northeast Asia, the cultivated *F. holotricha* Koehne in Europe, *F. pallisiae* Wilmott, native to southeastern Europe, *F. sogdiana* Bunge in central Asia, and *F. nigra* Marshall, an ash native to North America (USDA-ARS, 2009).

**SYMPTOMS**

Symptoms include wilting and blackish discoloration of leaves, premature shedding of leaves, dieback of shoots, twigs and branches, necrosis of bark tissue, discrete necrotic cankers in the bark, and a brownish to greyish discoloration of the inner bark and wood that often extends beyond the region of visible bark necrosis (Halmschlager and Kirisits, 2008; Johansson et al., 2009; Kowalski and Holdenrieder, 2009a). In inoculated saplings, mycelium has been observed in ray parenchyma, phloem fibers, and xylem vessels (Schumacher et al., 2009). The fungus has been isolated from asymptomatic roots of inoculated ash saplings (Schumacher et al., 2009), but has also been found in dead roots of trees (Kowalski, 2006).

**BIOLOGY AND ECOLOGY**

**Life cycle:** Both the conidial anamorph and the apothecial teleomorph have been described for this species but their roles in the life cycle and spread of the pathogen have not been determined.

Conidia are produced in culture (Kowalski, 2006; Halmschlager and Kirisits, 2008) and sporulation has been found on the surface of lesions on inoculated young trees, but is rarely observed in the field (Kowalski and Holdenrieder, 2009b). Production of conidia in culture is enhanced at low temperatures but some isolates do sporulate at 23 to 25 C (Halmschlager and Kirisits, 2008; Jankovský and Holdenrieder, 2009). Conditions for ascospore maturation and release are not known.

Apothecia are produced on leaf petioles on the ground during August and September in Poland (Kowalski and Holdenrieder, 2009b) and from August to October in Switzerland (Breitenbach and Kranzlin, 1984) but were also found occasionally on shoots of dead seedlings in nurseries (Kowalski and Holdenrieder, 2009b). Abiotic stresses considered to be associated with ash dieback are drought, frost and changing winter conditions (Schumacher et al., 2007), but, whether and how they interact with the existing fungus to cause the "new" disease or if there has been a change in the fungus, is not understood at this time. The teleomorph was already known to be widespread in Czechoslovakia (Jankovsky and Holdenrieder, 2009) and present in other parts of Europe (Breitenbach and Kranzlin, 1984; White, 1944; Dennis, 1956). Because canker growth has been observed to be greater in winter, the fungus appears to be adapted to cold weather (Jankovsky and Holdenrieder, 2009).

Differences in cultural morphology of isolates are illustrated in Halmschlager and Kirisits (2008). Ten isolates of *C. fraxinea*, most of them from Germany, varied in extracellular oxidase activity (Schumacher et al., 2009).

**Associations:** Other fungi, some of which may be opportunistic pathogens invading the lesions caused by *C. fraxinea*, are readily isolated from necrotic bark (Kowalski and Holdenrieder, 2009b; Schumacher et al., 2009). Root-infecting *Phytophthora* species were not found to be involved in the disease of ash dieback in Sweden (Bakys et al., 2009a; Schumacher et al., 2009).

**MOVEMENT AND DISPERSAL**

**Natural dispersal:** Conidia are produced in droplets or chains (Kowalski, 2006; Talgo et al., 2009). Described as "sticky" (Kowalski and Holdenrieder, 2009b), they would not appear to be adapted for airborne dispersal. Ascospores are dispersed through the air (Kowalski and Holdenrieder, 2009b), although the apothecia, so far found predominantly in the forest leaf litter, would not appear as well-placed for wide dissemination of spores as those of *Cenangium ferrugineum*, which are produced on dead twigs still attached to trees (Sinclair and Lyon, 2005). The observation by Kowalski and Holdenrieder (2009b) of apothecia on some dead sapling shoots suggests that they may also occur on above-ground parts of naturally infected trees.

**Vector transmission:** No vector is known (Kowalski and Holdenrieder, 2009b), but insects are known to have a role in dispersal of conidia of other *Chalara* species (Kile, 1993). Some of these fungi produce attractive volatile compounds on infected trees (Kile, 1993). Fungal spores produced in liquid droplets are adapted for insect dispersal, and some scolytid beetle larvae develop in leaf petioles in the litter layer under trees (Crowson, 1984).

**Accidental introduction:** The pathogen could be carried from nurseries in asymptomatic or overlooked infected saplings (Kirisits et al., 2009; Schumacher et al., 2009).
Economic impact: This fungus could cause losses of different severity depending on whether affected trees are in forests, planted as ornamentals, or raised in nurseries (Schumacher et al., 2009; Talgo et al., 2009). Kowalski (2006) reported that in Poland trees were killed in all age classes and regardless of site conditions.

PREVENTION

SPS measures (quarantine):

Because ash saplings may be infected without showing symptoms, quarantines may be necessary to prevent additional distribution from affected nurseries in Europe. Restriction of the movement of other ash material may be useful or necessary due to the possible role of vectors (NAPPO, 2009).

Importation of *Fraxinus* species from European countries to the USA was prohibited already due to the occurrence of another pathogen, *Pseudomonas savastanoi*, that causes cankers and dwarfing (CFR, 2008a), and importation of ash plants from other countries was later prohibited in order to prevent further introductions of the emerald ash borer (CFR, 2008b).

CONTROL

Cultural control and sanitary measures: Not enough is known of the biology of this pathogen to indicate the usefulness of particular methods. Avoidance of wounding and destruction of infected plants or plant parts are control measures suggested for other dieback and canker-causing fungi (Kile, 1993). Leaf scars have served as an infection courts for artificial inoculation of saplings (Talgo et al., 2009).

Host resistance: Individual one-year-old seedlings vary in susceptibility, suggesting the possibility of selecting resistant clones (Bakys et al., 2009a). Other ash species in the section *Fraxinus* or other sections could be tested as sources of resistance.

GAPS IN KNOWLEDGE/RESEARCH NEEDS

As Schumacher et al. (2009) indicate more research is needed concerning the source of inoculum, possible vectors, points of infection, and the conditions of the infection process itself. Information on these may yield clues concerning the reason for the emergence of this disease (Kowalski and Holdenrieder, 2009b; NAPPO, 2009).

Clones of the known susceptible species and other *Fraxinus* species should be tested by inoculation to identify sources of resistance as well as to establish the possible host range for the pathogen in Europe and elsewhere.

Use of molecular methods to test nursery trees for infection may validate the methods and provide data for determination of the need for quarantines to prevent spread of the fungus from nurseries.

References
Phialides, 1000x. CBS 122503: from malt extract agar.

Phialides and conidia, 1000x. CBS 122503: from malt extract agar.

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