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Full Length Research Paper

Association between *Hymenoscyphus pseudoalbidus* and Ash Dieback in Austrian Forest Nurseries

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Dieback of *Fraxinus excelsior, Fraxinus angustifolia* and other *Fraxinus* species is an emerging infectious disease caused by the ascomycete fungus *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*). Investigations in five forest nurseries in Austria from 2008 to 2011 showed that ash dieback is a common and important disease of nursery seedlings. *H. pseudoalbidus* was consistently isolated at high frequencies from symptomatic *F. excelsior* and *F. angustifolia* seedlings, confirming that this fungus is associated with ash dieback. Symptom observations on potted seedlings revealed progression of the disease outside the vegetation period and thus a long incubation period in the disease cycle of ash dieback. Apothecia of *H. pseudoalbidus* were occasionally observed on dead ligneous parts of common ash, including stems of nursery seedlings. This suggests that a low portion of diseased seedlings can initiate new infections, which may be important for moving the pathogen to new areas. Based on our observations and studies in forest nurseries and the present knowledge on the disease cycle of ash dieback, recommendations for disease management in tree nurseries and concerning artificial regeneration are presented.

Key words: *Chalara fraxinea*, *Fraxinus excelsior*, *Fraxinus angustifolia*, emerging forest disease, disease management.

INTRODUCTION

During the last two decades, ash dieback caused by the ascomycete fungus *Hymenoscyphus pseudoalbidus* (Figure 1; anamorph *Chalara fraxinea*; Kowalski, 2006; Kowalski and Holdenrieder, 2009a, b; Queloz et al., 2011) has successively emerged in many European countries (Husson et al., 2011; Timmermann et al., 2011). Everywhere the disease appears it causes im-mense damage to common ash (*Fraxinus excelsior*) and in some parts of Europe also to narrow-leaved ash (*Fraxinus angustifolia*) (Kowalski and Łukomska, 2005; Kirisits et al., 2009, 2010; Schumacher et al., 2010; Schumacher, 2011). In addition, ash dieback has also been reported on a few ash species not native to Europe (Drenkhan and Hanso, 2010).

Its gradual spread and the high disease intensity may indicate that *H. pseudoalbidus* is an invasive alien organism (Husson et al., 2011; Queloz et al., 2011; Timmermann et al., 2011). Trees of all ages, in the forest, the landscape and in urban environments, both naturally regenerated and planted ones, are affected (Kowalski and Łukomska, 2005; Kirisits et al., 2009; Schumacher, 2011). Moreover, the disease is an economically important problem in shade tree and forest nurseries (Kowalski and Łukomska, 2005; Schumacher et al., 2010; Schumacher, 2011). Here, we report on observations and investigations on ash dieback in forest nurseries in Austria.

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ASSOCIATION OF *H. PSEUDOALBIDUS* WITH ASH DIEBACK IN NURSERIES

Investigations on ash dieback were conducted from 2008 to 2011 in five forest nurseries. Seedlings were inspected for disease symptoms on the stem and on side twigs. From seedlings showing early symptoms of disease (rela-

Figure 1. Apothecia of the ash dieback pathogen *H. pseudoalbidus* on ash leaf petioles and rachises from the previous year in the forest litter. Note black pseudosclerotial plates (see also inset) on petioles and rachises, from which apothecia emerge. Ash trees are infected by airborne ascospores produced in the apothecia.

tively small necrotic lesions in the bark), fungal isolation was attempted. After surface sterilization (1 min in 96% ethanol, 3 min in 4% NaOCl, 30 s in 96% ethanol) of stem segments, the outer bark was carefully peeled off and small discs containing wood and phloem were cut near the transition zone between necrotic and healthy phloem and placed on malt extract agar (MEA; 20 g/L malt extract, 16 g/L agar, 100 mg/L streptomycin sulphate). The primary isolation plates were at first incu-bated at room temperature in diffuse daylight (nursery 1, in 2008), but later (nurseries 1 to 5, in 2009 and 2011) at cool temperatures (between 4 to 10°C) in the dark. The latter was done in order to stimulate anamorph pro-duction of *H. pseudoalbidus* and to give it competitive advantage over other fungi, thereby increasing the likelihood to detect the ash dieback pathogen (Kirisits et al., 2009). *H. pseudoalbidus* was identified based on morphological characteristics of its *Chalara fraxinea* stage (colony morphology, phialophores and spores). Other fungi were not determined.

Typical symptoms of ash dieback, including necrotic lesions in the bark and phloem, wood discoloration as well as dieback of shoots and entire seedlings were frequently observed in all five nurseries (Figure 2), on one-year-old to three-year- old ash seedlings. *H. pseu-doalbidus* was consistently isolated at high frequencies and often in pure culture from symptomatic *F. excelsior* seedlings in all five nurseries and in one nursery also from *F. angustifolia* seedlings. Overall, the ash dieback pathogen was obtained from 174 of the 192 (91%) investigated *F. excelsior* seedlings and from 14 of the 15 (93%) examined *F. angustifolia* plants. In agreement with a number of recent studies (for example Kowalski, 2006; Bakys et al., 2009; Kirisits et al., 2009, 2010; Kowalski and Holdenrieder, 2009a; Drenkhan and Hanso, 2010; Schumacher et al., 2010; Husson et al., 2011), the results clearly suggest that *H. pseudoalbidus* is associated with ash dieback and the cause of this disease.

DISEASE PROGRESSION OUTSIDE THE VEGETATION PERIOD

In mid-November 2010, three-year-old *F. excelsior* seedlings, rated as 'disease-free' based on external inspections were obtained from forest nursery 1. They were potted and placed in the garden of the institute. In spring 2011, the plants were again examined for the occurrence of ash dieback and isolations were done from diseased seedlings as described above. Upon inspection on 19 April 2011, 196 out of the 464 seedlings (42%) showed symptoms of ash dieback. The ash dieback pathogen was confirmed from all 41 plants (38 times in pure culture), from which isolations were made. As ascocarps of *H. pseudoalbidus* do not occur from October to May (Kirisits and Cech, 2009; Timmermann et al., 2011) and spores of *C. fraxinea* are most likely not infectious (Kirisits et al., 2009), the seedlings must have

Figure 2. Symptoms of ash dieback on seedlings of *F. excelsior*: (A) Necrotic bark lesion adjacent to a leaf scar, (B) Necrotic bark lesion with a small dead twig in the centre, (C and D) Extensive bark necrosis, (E) Package of ash seedlings prepared in autumn to be sold next year, containing diseased plants in spring.

been already infected by November, but were asymptomatic at that time and symptom progression took place outside the vegetation period. This is consistent with reports by forest nursery managers that considerable portions of apparently healthy ash seedlings, selected in autumn to be sold next year, were diseased when inspected again the following spring (Figure 2E). Disease progression during the cold period of the year is also consistent with the proposed disease cycle of ash dieback (Kirisits and Cech, 2009; Kirisits et al., 2009; Schumacher, 2011).

SPORULATION OF *H. PSEUDOALBIDUS* ON LIGNEOUS PARTS OF COMMON ASH

Structures resembling pseudosclerotial plates of *H. pseudoalbidus* were seen on stems, shoots and twigs of a few dead ash seedlings, in spring 2009 in nurseries 3 and 4 and in spring 2011 in nurseries 1 and 5, indicating that the fungus can probably form its apothecia on these seedlings later in the year. In summer 2011 stem segments of selected seedlings (from nursery 1) and natural regeneration as well as dead ash shoots and twigs with pseudosclerotial layers, collected from the forest litter at various localities, were placed in boxes filled with potting soil and incubated under moist conditions. Apothecia of *H. pseudoalbidus* subsequently developed, sometimes in high numbers (Figure 3), on a large portion of the incubated stems of nursery seedlings and naturally regenerated saplings as well as the ash shoots and twigs. This is consistent with previous reports on the occasional occurrence of apothecia on stems and shoots of one- to three-year -old dead nursery seedlings (Kowalski and Holdenrieder, 2009b) as well as on dead ash shoots and twigs in the forest litter (Kirisits and Cech, 2009). In contrast, teleomorph formation on ligneous tissues of ash is not known for *Hymenoscyphus albidus*, the long-known and apparently native sibling species of the ash dieback pathogen (Kowalski and Holdenrieder, 2009b; Queloz et al., 2011).

H. pseudoalbidus is effectively disseminating via its airborne ascospores that are primarily produced in apothecia on leaf petioles and rachises from the previous year on the ground (Figure 1; Kirisits and Cech, 2009; Kowalski and Holdenrieder, 2009b; Timmermann et al., 2011). This mode of dispersal likely explains the rapid recent spread of the fungus in Europe (Kowalski and Holdenrieder, 2009b; Timmermann et al., 2011). However, trade with infected nursery seedlings could have

Figure 3. Apothecia of *H. pseudoalbidus* on *F. excelsior* twigs. Note black pseudosclerotial layers that had formed underneath the epidermis on each of the twigs. The size of the largest apothecial disc flats shown on each of the photos is about 5 mm.

been and still may be an important pathway to introduce the pathogen into new areas and to accelerate its spread (Kirisits et al., 2010; Timmermann et al., 2011). The occasional observation of *H. pseudoalbidus* apothecia on ligneous tissues of common ash indicates that nursery seedlings can indeed be infectious. It is likely that the movement of apparently and latently diseased seedlings, on which inoculum subsequently develops, can lead to the initiation of new disease centres, even far away from natural infection sources. Likewise, infected ash leaf petioles and rachises, including those with pseudosclerotial plates from the litter, may be moved together with bare-root and container-grown plants (Timmermann et al., 2011).

RECOMMENDATIONS FOR DISEASE MANAGEMENT

Although the origin of *H. pseudoalbidus* remains enigmatic, the pathogen behaves as an invasive tree pathogen and is a serious threat to *F. excelsior* and other

Fraxinus species (Kowalski, 2006; Bakys et al., 2009; Kirisits et al., 2009, 2010; Drenkhan and Hanso, 2010; Schumacher et al., 2010; Husson et al., 2011; Queloz et al., 2011; Timmermann et al., 2011). The disease cycle of ash dieback is now understood sufficiently well (Kirisits and Cech, 2009; Kirisits et al., 2009; Kowalski and Holdenrieder, 2009b; Schumacher, 2011; Timmermann et al., 2011) that recommendations for disease management concerning seedlings and artificial regeneration can be given. Measures are grouped according to the strategies presented by Tainter and Baker (1996). As knowledge on ash dieback is still limited amongst many practitioners and stakeholders, especially in countries and areas, where the disease presently does not occur, raising awareness on the disease forms the basis for implementing disease management measures.

Exclusion

i. Plant quarantine measures for nursery seedlings (import bans, imports from confirmed disease-free areas only, plant inspections, plant passports and certifications) may be effective to avoid or delay the movement of the ash dieback pathogen to geographically isolated parts of Europe such as the British Isles and to other continents. However, the long inclubation period in the disease cycle of ash dieback makes inspection of ash plants extremely difficult.
ii. Import bans for nursery seedlings and possibly other commodities (for example ash logs and timber) and thus closing potentially dangerous pathways would be the most effective measures to avoid the movement of *H. pseudoalbidus*, but are difficult to enforce politically.

Avoidance

i. Due to the risks posed by the disease, wide afforestation of susceptible ash species (especially *F. excelsior* and *F. angustifolia*) is no longer recommended. Site adapted alternative species should be given preference. If ash is used to some degree, it should be planted in mixture with other species.
ii. Observations in a number of countries (for example Schumacher, 2011) suggest that disease intensity is lower on drier sites within the ecological amplitude of *F. excelsior*. Still using ash on such sites may thus be justified, as well as in situations where wood production is not the main management goal, for example in shelterbelts, landscape and urban plantings.
iii. Flowering ash (*Fraxinus ornus*) that has so far not been affected by ash dieback may in some situations be an alternative.
iv. Little is known on the susceptibility of non-native *Fraxinus* species to the disease, but Drenkhan and Hanso (2010) reported ash dieback and confirmed the occurrence of *H. pseudoalbidus* on *F. americana*, *F. nigra* and *F. pennsylvanica* from North America and *F. mandshurica* from Asia. Exotic ash species should thus be used only with caution as alternative to native *Fraxinus* spp.
v. As sporulation of and infection by *H. pseudoalbidus* is favoured by high soil and air humidity, ash seedling beds should be established at sites with fairly dry microclimate, offering the prospect of lower disease levels.
vi. In order to decrease infections, seedling beds should be located large distances away from natural inoculum sources in the surroundings.
vii. Both producers and buyers should carefully inspect nursery plants for the occurrence of ash dieback. It is recommended that users continue the inspections after trees have been planted. If plants have been unambiguously infected in the nursery, buyers can claim for compensation.
viii. Many seedlings appear to be disease-free in autumn, but develop externally visible symptoms during winter and early spring. Purchasing trees in spring therefore increases the chance to obtain mostly healthy plants.

Inoculum reduction

i. The removal and destruction of shed ash leaves may in some situations be economically feasible to reduce inoculum and thus to decrease infections in nurseries. Leaves should be burnt, buried, ploughed into nursery beds or covered with soil.
ii. Routine removal of leaves in cities, in addition to dry climatic conditions in urban environments, not very conducive for inoculum production of and infection by *H. pseudoalbidus*, may explain why ash dieback is presently often of relatively minor importance on shade, avenue and park trees.

Protection

Fungicide treatments may be an option in nurseries, however, as the infectious period of *H. pseudoalbidus* is long (mid-June to early September in Central Europe) many applications are likely necessary. Overall, fungicide treatments will be of limited value to manage ash dieback, as seedlings remain susceptible when planted in the field.

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