

# Further Observations on the Association of *Hymenoscyphus fraxineus* with *Fraxinus ornus*

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## Abstract

Results of ash dieback symptom observations on 15 seedlings each of *Fraxinus ornus* (flowering ash) and *Fraxinus excelsior* (common ash) which were planted on a forest site in south-eastern Austria where they were exposed to natural inoculum (ascospores) of the ash dieback pathogen *Hymenoscyphus fraxineus* in the growing seasons of 2014 and 2015 are presented. During the observations, until early March 2016 (572-845 days after the seedlings had been planted), necrotic lesions in the bark and wood discoloration, alone or in combination with leaf wilting and/or dieback due to girdling, were cumulatively recorded on all 15 and leaf symptoms associated with *H. fraxineus* on 14 out of the 15 *F. excelsior* seedlings. In contrast, no ash dieback symptoms on woody parts occurred on any of the *F. ornus* seedlings. In autumn 2014 and 2015, a few flowering ash leaves with inconspicuous necrotic lesions on the leaf rachis were observed, and *H. fraxineus* was isolated from 9 out of 20 (45%) symptomatic rachises, and once also from a necrotic leaflet midrib. The observations and fungal isolations confirm and strengthen previous appraisals that *F. ornus* is a host of *H. fraxineus*, that the fungus's occurrence on this ash species is presumably limited to leaves, on which it can complete its life cycle, and that its impact on flowering ash may be low. *F. ornus* is considered to be a highly resistant or tolerant host of *H. fraxineus*, despite it is not known to have undergone co-evolution with this otherwise so damaging invasive alien pathogen.

**Key words:** *Hymenoscyphus pseudoalbidus*, *Chalara fraxinea*, flowering ash, manna ash, ash dieback

## Introduction

Two of the three native European ash species, *Fraxinus excelsior* (common ash) and *Fraxinus angustifolia* (narrow-leaved ash) are highly susceptible to ash dieback (Bakys et al. 2009, Kirisits et al. 2009, 2010, Matlakova 2009, McKinney et al. 2014, Treitler 2014, Hauptman et al. 2016, Havdová et al. 2016), whereas relatively little is known about the association of *Hymenoscyphus fraxineus* with *Fraxinus ornus* (flowering or manna ash). There are no unambiguously confirmed reports that *H. fraxinus* can damage woody parts of flowering ash (e. g. Wallmann and Stingl 2011, Lösing 2013, Kirisits and Schwanda 2015). However, the fungus was recently isolated from leaf rachises with necrotic lesions (Kirisits and Schwanda 2015) and detected with real-time qPCR from asymptomatic leaf

blades of *F. ornus* (Schlegel et al. 2016.) Likewise, it was observed to form apothecia on pseudosclerotial leaf rachises and leaflet veins of this ash species in the leaf litter (Gross et al. 2014, O. Holdenrieder, personal communication). The first report of natural infection of *F. ornus* by *H. fraxineus* was based on isolation of the fungus from just four symptomatic leaves (Kirisits and Schwanda 2015). The aim of the present paper is to present further observations on symptoms caused by *H. fraxineus* on flowering ash and on the susceptibility of this ash species to this invasive alien pathogen. A particular purpose is to show photos of symptomatic flowering ash leaves from which *H. fraxineus* was isolated. Based on the present and previous studies, the potential impact of ash dieback on *F. ornus* is appraised and scientific and practical implications are discussed.

## Materials and Methods

Observations on the association of *H. fraxineus* with *F. ornus* were made on 15 seedlings of this ash species which had been planted (eight on 16 November 2013, six on 30 March 2014 and one on 16 August 2014) under the canopy of *Picea abies* trees and *Alnus glutinosa* coppice trees at a forest site in Stinatz (Austria, province of Burgenland, 47°12'42.5" N, 16°07'52.9" E, 300 m asl.), outside the natural range of *F. ornus*, where they were exposed to natural inoculum (ascospores) of the ash dieback pathogen. Fifteen seedlings of the highly susceptible *F. excelsior* were planted (six on 16 November 2013, four on 30 March 2014 and five on 16 August 2014) as positive controls along with the *F. ornus* plants. The observations were made on the same seedlings which had been investigated for the first definite report of *H. fraxineus* on *F. ornus* by Kirisits and Schwanda (2015), and further information on the seedlings, the site and the experimental planting can be found there. In summer 2015, two Norway spruce trees on the experimental site were infested and killed by the spruce bark beetle *Ips typographus*. In order to avoid further economic losses, all mature spruce specimens except one were harvested and removed in December 2015; therefore, the seedlings have since then been exposed to more open growing conditions.

The *F. ornus* and *F. excelsior* seedlings were visually inspected for symptoms of ash dieback on the main stem and on side twigs at six dates: 8 November 2014, 24 January, 20 June, 3-4 and 24 October 2015, as well as 10 March 2016. At four dates (8 November 2014, 20 June, 3-4 and 24 October 2015) the plants were also inspected for leaf symptoms. At all inspections symptomatic stem or twig samples (only from *F. excelsior*) and at the inspections on 8 November 2014, 3-4 and 24 October 2015 symptomatic leaf samples (only from *F. ornus*), which were still attached to the seedlings upon collection, were collected and transported to the laboratory, where they were subjected to fungal isolation. In the course of sampling, it was attempted to remove all visible necrotic parts of the stem and symptomatic twigs from affected *F. excelsior* seedlings, in order to free them from the disease. This was done to increase the chance that the plants survive infections, so that they remained available for later inspections.

Fungal isolations were made onto malt extract agar (MEA; 20 g DiaMalt malt extract (Hefe Schweiz AG, Stettfurt, Switzerland), 16 g Becoagar agar (W. Behrens & Co., Hamburg, Germany), 1000 ml tap water, 100 mg streptomycin sulphate (Calbiochem; Merck KGaA, Darmstadt, Germany), added after autoclaving) in 5.2-cm-diameter Petri dishes. From stems and twigs of *F. excelsior* seedlings, 5- to 8-cm-long segments containing, if possible, the transition between necrotic and healthy tissues were cut and subjected to surface sterilization (1 minute in 96% ethanol,

3 minutes in 4% NaClO, 30 seconds in 96% ethanol). The surface sterilized samples were allowed to dry for a few minutes. Thereafter, the outer bark was peeled off from the segments with a sterile scalpel, and 5- to 8-mm-wide discs containing wood and phloem were cut with sterile garden scissors at or near the transition zone between necrotic and healthy phloem or wood (except in the case of entirely necrotic woody parts, where only necrotic samples could be taken). Four discs per stem or twig were placed together in one Petri dish.

For 5- to 8-cm-long leaf rachis sections and leaflets (with necrotic midribs or necrotic dots) of *F. ornus* times of surface sterilization were shortened (30 seconds in 96% ethanol, 2 minutes in 4% NaClO, 30 seconds in 96% ethanol). From leaf rachises the epidermis was peeled off with a sterile scalpel after surface sterilization, but from leaflet midribs the epidermis could only partially be removed or not at all. Care was taken, however, to cut away surrounding parts of the leaf blade as best as possible. Depending on the extension of necrosis, 5- to 10-mm-long fragments from leaf rachises and leaflet midribs were taken at or near the transition between necrotic and healthy tissues or from entirely necrotic tissues. Four to 10 fragments per leaf rachis and four fragments per leaflet midrib were sampled; and four to five fragments from the same rachis or leaflet midrib were placed together in one Petri dish. From leaflets, discs containing necrotic dots were punched out with a sterile 5-mm-diameter cork borer. Three to four leaf discs per leaflet were placed together onto one MEA plate.

The primary isolation plates were incubated either at room temperature and diffuse daylight or at low temperatures (4-6 °C) in the dark, and repeatedly inspected for the growth of microorganisms during a period between six and eight weeks after isolation. *H. fraxineus* was determined based on its colony morphology and morphological characteristics of its asexual stage (Gross et al. 2014). For the first report of *H. fraxineus* on *F. ornus* (Kirisits and Schwanda 2015), the identity of two isolates was verified by ITS rDNA sequencing (GenBank accession numbers: isolate ST/FO/BS/2-1 = CBS 139781, KP994899; isolate ST/FO/BS/3 = CBS 139782, KP994900), following the methods described by Schwanda and Kirisits (2016), but the newly recovered isolates were solely determined morphologically. The isolation of other fungi was recorded, but they were not identified.

Kirisits and Schwanda (2015) reported the symptom observations on and isolation results from the *F. excelsior* and *F. ornus* seedlings at the site in Stinatz up to the inspection on 24 January 2015. These earlier results are incorporated in the present paper, and they are complemented with observations and isolations conducted until March 2016.

## Results

During the observation period, until 10 March 2016 (572-845 days (about 82-121 weeks) after the seedlings had been planted), symptoms of ash dieback on woody parts (necrotic lesions in the bark and wood discoloration, alone or in combination with leaf wilting and/or dieback because of stem or twig girdling) were cumulatively recorded on all 15 *F. excelsior* seedlings, of which three (20%) died due to the disease. On 13 plants (87%) symptoms occurred on the stem (on nine of these additionally also on side twigs), and on two (13%) only on side twigs. *H. fraxineus* was isolated from 13 of 14 diseased seedlings (93%) from which isolations were made; from nine it was obtained in pure culture and from four in mixed culture with other fungi (Table 1). In contrast to *F. excelsior*, no ash dieback symptoms on the main stem or on side twigs were observed on any of the 15 *F. ornus* seedlings at any of the six inspection dates.

At the inspections in autumn 2014 and 2015, leaf symptoms (necrotic lesions on rachises and leaflet veins) previously found to be associated with *H. fraxineus* (Bakys et al. 2009, Krätzler and Kirisits 2012, Gross et al. 2014, Steinböck 2014, Schwanda and Kirisits 2016) were frequently observed on *F. excelsior*. In November 2014, 12 of the 15 seedlings (80%) showed such symptoms (the remaining three were already fully defoliated; Kirisits and Schwanda 2015), while in October 2015, symptomatic leaves were recorded on ten of the 12 plants (83%) that were still alive. Considering the autumn assessments in both years, 14 of 15 seedlings (93%) were affected by leaf symptoms in at least one year, while the remaining seedling had shed all its leaves in November 2014 and was already dead at the inspections in October 2015.

At the inspection on 8 November 2014, all *F. ornus* seedlings except the one planted in August 2014 were

abundantly affected by leaf symptoms due to various, largely unknown causes, particularly on leaflets, and *H. fraxineus* was (rather unexpectedly) isolated from four leaf rachises (from one in pure culture) with inconspicuous necrotic lesions derived from leaves that were still attached to the plants at the time of collection (see Kirisits and Schwanda (2015) for further details). At the inspection on 24 October 2015, all seedlings except one displayed again various types of leaf symptoms at high frequencies. The most common symptoms were nearly circular or irregularly shaped necrotic lesions or blotches on leaflets presumably caused by the gall midge *Dasineura fraxinea* (Figure 1E-G; Schwerdtfeger 1981). As in the previous year, a few necrotic lesions on leaf rachises (Figure 1A-C) and in one case on a leaflet midrib (Figure 1D), which resembled symptoms occurring in connection with ash dieback on *F. excelsior* (Bakys et al. 2009, Krätzler and Kirisits 2012, Gross et al. 2014, Steinböck 2014, Schwanda and Kirisits 2016), were observed. *H. fraxineus* was isolated from 5 of 16 rachises (31%) with necrotic lesions (twice in pure culture; examples of rachises with lesions from which the fungus was isolated are shown in Fig. 1A-C) and in mixed culture with other fungi from the necrotic leaflet midrib just mentioned before (Fig. 1D). Isolations were also done from two other necrotic midribs (one associated with damage by *D. fraxinea*) but *H. fraxineus* was not recovered from them (Table 1). Isolation frequencies from leaf rachises summarized for November 2014 and October 2015 are shown in Table 1. Another symptom observed were small necrotic dots on leaflets (Fig. 1H), which were similar to those caused by *H. fraxineus* on *F. excelsior* (Gross et al. 2014, Steinböck 2014). Attempts to isolate the ash dieback pathogen from leaf blade tissues showing such symptoms were, however, not successful (Table 1).

**Table 1.** Frequencies of *Hymenoscyphus fraxineus* (Hf) and other fungi (Of) isolated from 2014 to 2016 from stems and twigs of *Fraxinus excelsior* with necrotic lesions in the bark and wood discoloration, and in autumn 2014 and 2015 from different parts of symptomatic *Fraxinus ornus* leaves (leaf rachises and leaflet midribs with necrotic lesions, as well as leaf blades with necrotic dots) at the experimental planting in Stinatz (Austria)

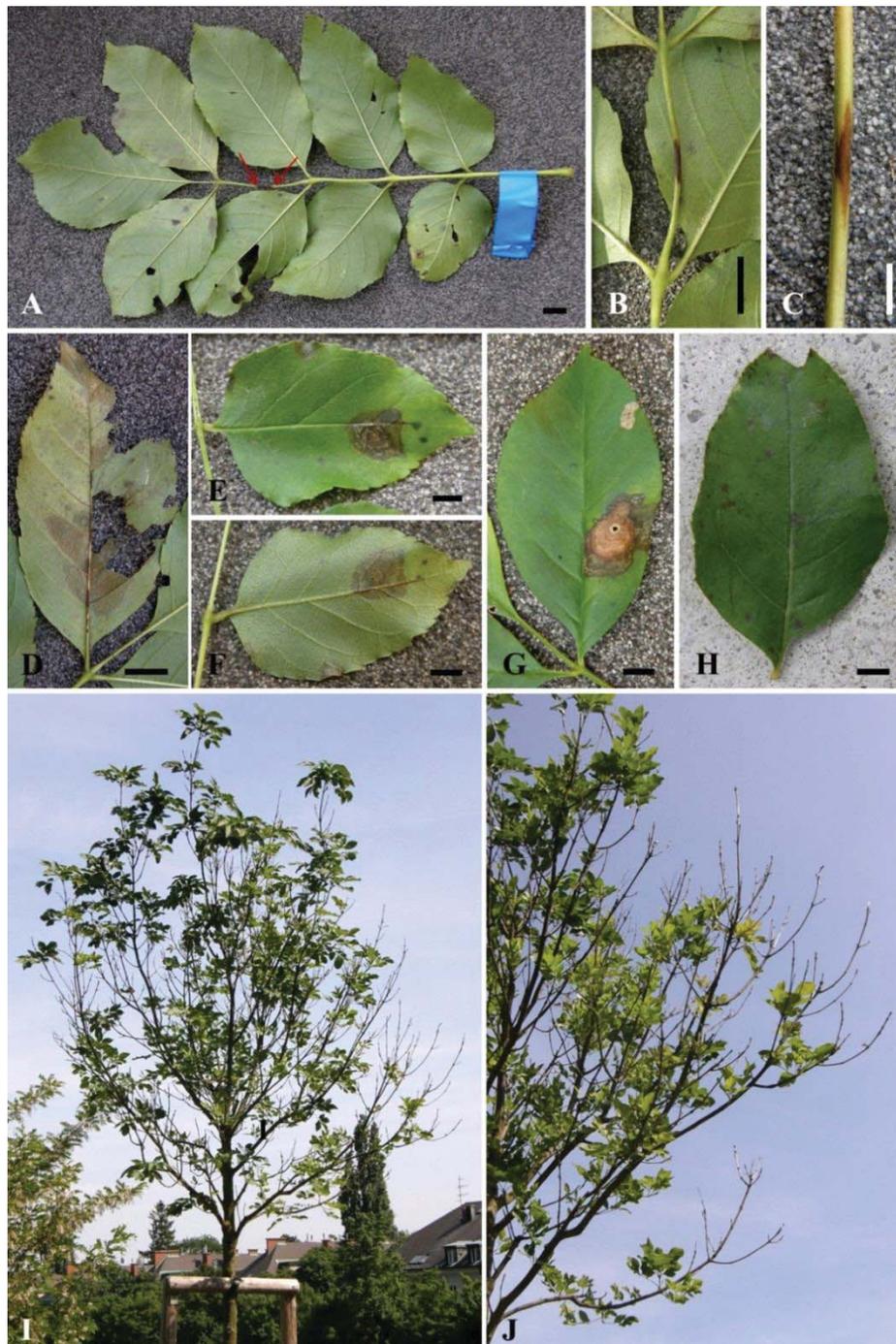
<i>Fraxinus</i> species and plant part	Sample size (N) and isolation frequency (%) <sup>1</sup>						Sterile
	N	Hf (total)	Only Hf	Hf and Of	Only Of	Of (total)	
<i>F. excelsior</i> , stems and twigs	14 <sup>2</sup>	93	64	29	7	36	0
<i>F. ornus</i> , leaf rachises	20 <sup>3</sup>	45	15	30	55	85	0
<i>F. ornus</i> , leaflet midribs	3 <sup>4</sup>	33	0	33	67	100	0
<i>F. ornus</i> , leaf blades	31 <sup>4</sup>	0	0	0	39	39	61

<sup>1</sup>For leaf rachises and leaflet midribs percentages refer to samples from which *H. fraxineus* and other fungi were isolated from at least one fragment per rachis or midrib. For leaf blades percentages refer to individual fragments / discs taken from areas of leaflets with necrotic dots (three to four were taken from each of eight leaflets from two trees). Other fungi were not identified. Sterile refers to leaf blade samples yielding no fungal growth.

<sup>2</sup>Isolations were made from samples collected on the following dates (number of seedlings in parentheses): 8 November 2014 (1); 24 January (5), 20 June (3), 3-4 October (3), 24 October 2015 (1); and 10 March 2016 (1).

<sup>3</sup>Isolations were made from four leaf rachises collected on 8 November 2014 (from all of which *H. fraxinus* was isolated; Kirisits and Schwanda 2015) and from 16 leaf rachises collected from seven different trees on 3-4 and 24 October 2015.

<sup>4</sup>Isolations were made from leaves collected on 24 October 2015.



**Figure 1.** A-H, examples of leaf symptoms on *Fraxinus ornus* at the experimental planting in Stinatz at the inspection on 24 October 2015: A, a leaf with a small, inconspicuous necrotic lesion (indicated with red arrows) on the rachis, shown in (B) at higher magnification, C, another necrotic rachis lesion, D, a leaflet with mechanical damage and necrosis of the midrib, E-G, necrotic lesions and blotches on leaflets presumably caused by the gall midge *Dasineura fraxinea* (E upper side and F lower side of the same leaflet), H, a leaflet with small necrotic dots on the leaf blade; scale bars: 10 mm (A, B, D), 5 mm (C, E, F, G, H); *Hymenoscyphus fraxineus* was isolated in mixed culture with other fungi from the necrotic rachis lesions shown in A and B, and C, as well as from the necrotic leaflet midrib shown in D; I-J, shoot and twig dieback resembling symptoms of ash dieback on a shade tree of *Fraxinus ornus* in an alley in Vienna (Dornbach, 21 May 2009); *Hymenoscyphus fraxineus* was not isolated from any symptomatic shoot or twig of this tree, and the damage was suspected to be due to other causes (probably intensive flowering in the previous year, frost or abiotic stress because of recent planting of the tree)

## Discussion

The observations and isolation results reported here confirm and strengthen the appraisals by Kirisits and Schwanda (2015) that *F. ornus* is a host of *H. fraxineus*, that the occurrence of the fungus on this ash species is presumably limited to leaves and that its impact on flowering ash may be low, in contrast to *F. excelsior* and *F. angustifolia* which are severely damaged by ash dieback (Bakys et al. 2009, Kirisits et al. 2009, 2010, Matlakova 2009, McKinney et al. 2014, Treitler 2014, Hauptman et al. 2016, Havdová et al. 2016, Schwanda and Kirisits 2016). The high incidence of ash dieback on the *F. excelsior* seedlings and the mortality of a portion of the plants (despite visually recognizable necrotic parts were removed during inspections) indicate a considerable infection pressure by *H. fraxineus* at the experimental site in Stinatz. Despite this, no ash dieback symptoms on the stem or on side twigs of the *F. ornus* seedlings were recorded.

Careful inspection of just 15 seedlings during about 16 months may be viewed as insufficient to definitely conclude that *H. fraxineus* does not damage woody parts of *F. ornus*. However, this conclusion is supported by the absence of ash dieback on *F. ornus* during inspections in Austria, in forests and on shade trees (Kirisits 2008, Wallmann and Stingl 2011), in a nursery planting experiment (T. Kirisits, C. Gartlehner, C. Lamberg and H. Konrad, unpublished data) as well as on potted plants used for inoculation experiments in the institute's garden (K. Schwanda and T. Kirisits, unpublished observations). In all these situations, at least a few and often many plants of adjacently growing susceptible ash species (*F. excelsior* and sometimes also *F. angustifolia*) showed necrotic lesions in the bark and shoot dieback. Likewise, unambiguous ash dieback symptoms on flowering ash have as yet not been reported from other European countries, including Germany (Lösing 2013), Italy (Luchi et al. 2012, Kirisits and Schwanda 2015, Ghelardini et al. 2017), Slovakia (Longauerová et al. 2012), Slovenia (Hauptman et al. 2012) and Switzerland (Engesser and Meier 2012). Cankers and dieback on *F. ornus* reported by Lehtijärvi et al. (2009) from Turkey and twig dieback by Kopinga and de Vries (2017) from Amsterdam (the Netherlands) were likely caused by other agents; symptoms were not associated with *H. fraxineus* in the former study and pathogen detection was not done in the latter study. At one instance, in May 2009, shoot dieback closely resembling ash dieback was observed on flowering ash trees growing in an alley in Vienna (Figure 1I-J), but *H. fraxineus* was not isolated, which emphasizes that such symptoms can have many causes. In the future, crown dieback symptoms on *F. ornus* should be viewed with caution, and not considered to be caused by *H. fraxineus* unless its involvement is proven by isolation or molecular detection.

In the autumn of both years, foliage of the *F. ornus* seedlings was rarely and negligible affected by necrotic lesions on the rachis and on leaflet veins, the symptoms *H. fraxineus* could be partially linked to. The fungus's low isolation frequency in 2015 may be due to its slow growth, making its detection by isolation onto agar media difficult. It is also possible that other fungi (e. g. some of those mentioned by Schlegel et al. (2016)) can cause necrotic lesions on leaf rachises, particularly on senescent foliage late in the growing season when the inspections were made. Foliage of the *F. excelsior* seedlings at the site in Stinatz was generally more frequently and more severely affected by necrotic lesions on rachises and leaflet veins (shown to be caused by *H. fraxineus* in previous studies; Bakys et al. 2009, Kräutler and Kirisits 2012, Gross et al. 2014, Steinböck 2014, Schwanda and Kirisits 2016). This indicates, in agreement with the total absence of shoot symptoms, that *F. ornus* leaves are highly resistant or tolerant to *H. fraxineus*, respectively that the fungus displays only a low level of virulence to leaves of this ash species. This view is consistent with wound inoculation experiments on leaves, in which *H. fraxineus* was pathogenic to *F. ornus*, but more virulent to *F. excelsior* and *F. angustifolia*, respectively the two latter ash species were more susceptible than the former one (Schwanda and Kirisits 2016). In line with these results, *H. fraxineus* grows significantly slower on agar media amended with leaves of *F. ornus* than on media amended with leaves of *F. excelsior* and *F. angustifolia* (Carrari et al. 2015).

The scarce occurrence of symptoms caused by *H. fraxineus* on *F. ornus* leaves late in the growing season could indicate that the fungus behaves mainly as an endophyte on this ash species, because endophytes often cause symptoms on senescent foliage towards the end of the growing season. Alternatively, the low incidence of symptoms may be because *H. fraxineus* is a relatively rare colonizer of flowering ash leaves. In a study in Switzerland, the ash dieback pathogen was not isolated, but scarcely detected with real-time qPCR from symptomless leaflets (Schlegel et al. 2016). The behaviour and colonization profile of *H. fraxineus* in flowering ash leaves (see Hietala et al. (2013) and Steinböck (2014) for *F. excelsior*) and whether the fungus is associated with other leaf symptoms such as necrotic dots on leaflets observed in this study (Fig. 1H) require further investigation.

In the present study, leaf debris in the litter was not inspected for apothecia of *H. fraxineus*, but fruiting bodies were observed on naturally infected pseudosclerotial leaf rachises and leaflet veins of *F. ornus* in Switzerland (Gross et al. 2014, O. Holdenrieder, personal communication) and Austria (T. Kirisits, unpublished observations), which indicates that the fungus can complete its life cycle on leaves of this ash species. In contrast, apothecia of *Hymenoscyphus albidus* are not known to occur on flowering ash (Baral and

Bemmann 2014); it may therefore not be a host of this long-known native European sibling species of *H. fraxineus*.

All available evidence suggests that *F. ornus* is highly resistant or tolerant to *H. fraxineus* (see Landolt et al. (2016) for a discussion of resistance and tolerance in the context of ash dieback), unless the fungus changes its behaviour. Such a behavioural change could be caused by the introduction of more virulent strains of the pathogen from Asia, as discussed by Gross and Sieber (2016). In wound inoculation trials, *H. fraxineus* incited necrotic lesions and wood discoloration on stems of flowering ash seedlings (Kirisits et al. 2009, Matlakova 2009), which indicates that under natural conditions defence mechanisms on and in leaves or in the leaf scar region prevent the fungus to progress into shoots. Intriguingly, *F. ornus* may be more resistant to ash dieback than *Fraxinus mandshurica* (Manchurian ash), one of the natural hosts of *H. fraxineus* in Asia. On *F. mandshurica*, *H. fraxineus* was originally thought to occur as a harmless leaf endophyte (Gross et al. 2014, Cleary et al. 2016), but Drenkhan et al. (2017) recently reported that the fungus is associated with frequently occurring leaf symptoms on this ash species in its native range in Far East Russia. *H. fraxineus* also causes symptoms on both leaves and shoots of introduced Manchurian ash in Estonia (Drenkhan and Hanso 2010). Moreover, in wound inoculation experiments it incited necrotic lesions in the bark on *F. mandshurica* var. *japonica* seedlings (Gross and Holdener 2015).

Within the genus *Fraxinus*, *F. ornus* belongs to the section *Ornus* (consisting of species occurring in Eurasia), in contrast to *F. excelsior* and *F. angustifolia*, which form part of the section *Fraxinus* (with species distributed in Eurasia and North America) (Wallander 2008). Flowering ash is the first species in the section *Ornus* known to be a host of *H. fraxineus* outside the pathogen's natural range. The high resistance or tolerance of this ash species to ash dieback is despite it is not known to have undergone co-evolution with *H. fraxineus*, which is so damaging to its two other European hosts, *F. excelsior* and *F. angustifolia*. Likewise, the low susceptibility of *F. ornus* to *H. fraxineus* can likely not be explained by co-evolution with *H. albidus*, which has, as mentioned already, so far not been recorded on this ash species. One of the known natural hosts of *H. fraxineus* in Asia, *F. rhynchophylla* or *F. chinensis* subsp. *rhynchophylla* (Korean ash; Han et al. 2014, Gross and Han 2015; treated as a synonym of *F. chinensis* by Wallander (2008)) also forms part of the section *Ornus*, in contrast to *F. mandshurica* which belongs to the section *Fraxinus* (Wallander 2008). This may reflect a shared evolutionary history of *F. ornus* with an Asian host of *H. fraxineus* in the section *Ornus*, which left resistance genes and traits to the fungus in the former ash species. However, in Far East Russia *F. rhynchophylla* was affected by leaf symptoms associated with *H. fraxineus*, suggesting that the

fungus is a pathogen on this ash species as well (Drenkhan et al. 2017). Present evidence may suggest that the susceptibility of various ash species to *H. fraxineus* is related to their phylogenetic position within the genus *Fraxinus*, with species in the section *Fraxinus* outside the pathogen's natural range (*F. angustifolia*, *F. excelsior*, *F. nigra*) being highly susceptible, those in the section *Ornus* (*F. ornus*) showing low susceptibility and species in the section *Melioides* (distributed in North and Central America; *F. americana*, *F. pennsylvanica*) displaying intermediate susceptibility (Kirisits et al. 2009, 2010, Matlakova 2009, Drenkhan and Hanso 2010, Lösing 2013, Gross et al. 2014, McKinney et al. 2014, Treitler 2014, Gross and Sieber 2016, Kowalski et al. 2015, Hauptman et al. 2016, Havdová et al. 2016, Schwanda and Kirisits 2016). However, knowledge on more ash species is necessary to definitely infer on the relationship between the phylogeny of various *Fraxinus* spp. and their susceptibility to *H. fraxineus*.

Based on the present state of knowledge, *H. fraxineus* will likely not have a serious impact on *F. ornus* in natural and managed ecosystems and not impair its future use as forest and ornamental tree. In fact, the further invasion of the fungus into the natural distribution range of flowering ash (from which it is presently largely absent) may occur inconspicuously (if it does not co-occur with one or both of the other, susceptible, European ash species) because symptoms of infections are not striking and difficult to detect. In the search for the genetic background of resistance or susceptibility to *H. fraxineus* within the genus *Fraxinus*, flowering ash can be viewed as a "model" species of a highly resistant host (Harper et al. 2016). The findings presented here have also quarantine implications because *H. fraxineus* may be moved to new areas on plants with leaves or with pseudosclerotial leaf parts of *F. ornus*, a risk which can be greatly reduced if only bare-rooted material from which any accompanying leaf debris is removed is traded. Finally, the example of *F. ornus* emphasizes that exposing plants to high loads of ascospores under field, semi-field or laboratory conditions is presumably the best way to study the susceptibility of ash species to *H. fraxineus*, the symptomatology of ash dieback on various hosts and the infection biology of the pathogen (Cleary et al. 2013, Kirisits and Schwanda 2015).

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