Fungal Endophytes in Ash Shoots – Diversity and Inhibition of *Hymenoscyphus fraxineus*

ZUZANA HAŇÁČKOVÁ[1,2]*, LUDMILA HAVRDOVÁ[1], KAREL ČERNÝ[1], DANIEL ZAHRADNÍK[1] AND ONDŘEJ KOUKOL[2]

[1]*Department of Biological Risks, Silva Tarouca Research Institute for Landscape and Ornamental Gardening Publ. Res. Inst., Květnové náměstí 391, Průhonice 252 43, Czech Republic*

[2]*Department of Botany, Faculty of Science, Charles University, Benátská 2, Prague 128 01, Czech Republic*

[*]*Corresponding author: E-mail: Zuzana.Hanackova@vukoz.cz, tel. +420 296528368*

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Abstract

The population of European ash (*Fraxinus excelsior*) in Europe is severely affected by ash dieback disease caused by *Hymenoscyphus fraxineus*. Endophytic fungi are known to influence tree fitness and there are efforts to use them directly or indirectly in the biological control of tree pathogens. To assess possible variation in the fungal community depending on the health status of the tree, three pairs of ash dieback relatively resistant and ash dieback susceptible adult trees were selected from two locations. The diversity of fungal endophytes in healthy ash shoots was investigated in the summer and winter season by agar culture isolations. To screen for the antagonistic potential of ash endophytes to *H. fraxineus*, 48 isolated taxa were tested in dual cultures with the pathogen. Distinctive seasonal changes were observed in the identified fungal communities. Endophytes with a presumptive saprotrophic functional role increased in the summer, whereas presumptive pathogenic taxa increased in winter. Furthermore, species diversity was significantly higher in the winter. Higher frequencies of *Diaporthe* sp. 1 and *Diaporthe* sp. 2 were recorded in susceptible trees than in resistant trees. However, no significant differences were found between community structures. The growth of *H. fraxineus* was significantly reduced by 36 endophytes, with inhibition rates ranging from 42 to 83%. The best inhibition results were obtained for fast growing fungi such as *Botrytis cinerea* and *Phoma macrostoma* var. *incolorata*.

Key words: *Fraxinus excelsior*, endophyte community, dual cultures, antagonism, *Hymenoscyphus fraxineus*

Introduction

The recent spread of *Hymenoscyphus fraxineus*, the causal agent of ash dieback, resulted in a substantial threat to native ash stands and forestry (McKinney et al. 2014, Landolt et al. 2016). Several European countries with a high ratio of ash in forestry production reported high losses of adult trees (Pliūra et al. 2011, McKinney et al. 2014). Artificial re-establishment of ash stands is no longer recommended due to the high probability of disease return (Pliūra et al. 2014). Therefore, interest among practitioners to plant the species has been limited (McKinney et al. 2014). Moreover, the production of ash seedlings in nurseries has also been affected due to the effective spread of the pathogen and the lack of customer interest (Havrdová et al. this publication).

Some ash trees show less susceptibility to ash dieback than others; a heavily damaged tree may be observed in proximity to a tree having almost no signs of dieback. These differences have been connected with the genetic variability of individual trees (Pliūra et al. 2011, McKinney et al. 2012, Stener 2013, Lobo et al. 2015). However, tree genotype, along with tree physiology, can also influence the endophytic

fungal community (Rajala et al. 2013, Rajala et al. 2014). Tree pathogen resistance can even lead to thorough reduction of endophytic colonization in target tissue (Martín et al. 2013). Endophytes are known for their defensive power against various tree pathogens. Although a supportive role has been attributed to the fungal communities of trees (Arnold et al. 2003, Ganley et al. 2008, Mejía et al. 2008, Witzell et al. 2014), there are also concerns about isolates of fungal species having the opposite function; some of them may contribute to the development of tree diseases (Bengtsson et al. 2014).

Fungal endophytes and saprotrophs of European ash (*Fraxinus excelsior*) have previously been investigated in leaves (Unterseher et al. 2007, Scholtysik et al. 2013, Cross et al. 2016), living branches (Kowalski and Kehr 1992) and attached dead branches (Butin and Kowalski 1986, Griffith and Boddy 1991, Unterseher et al. 2005, Unterseher and Tal 2006). An intensive search for the causal agent of ash dieback led to surveys in the past decade of fungi in ash shoots, branches, roots, buds, litter petioles and seeds (Przybył 2002, Kowalski and Łukomska 2005, Bakys et al. 2009a, Chen 2012, Cleary et al. 2013, Davydenko et al. 2013). In addition to *H. fraxineus*, the results indicated the presence of other potential pathogens, but pathogenicity to ash was confirmed only for a few of the tested species (Przybył 2002, Bakys et al. 2009a).

Mechanisms by which endophytes can influence the presence of pathogens in a tree consist of direct effects including antibiosis by metabolites, competition for nutrients or space and mycoparasitism, and indirect effects including induced systemic resistance of the tree (Viterbo et al. 2007, Lacava and Azevedo 2014). Preliminary tests of antagonism *in vitro* are beneficial in searching for potential biological control agents with direct effects on unwanted fungi (Mejía et al. 2008). Such screening may also reveal a possible inverse impact of the pathogen on native fungal species. The first studies with *H. fraxineus* showed that it created an inhibition zone in the presence of other fungi *in vitro* (Kowalski and Holdenrieder 2009, Kowalski and Bartnik 2010). Schulz et al. (2015) reported mutual antibiosis between *H. fraxineus* and ash endophytes resulting in reduced concentrations of phytotoxins produced by *H. fraxineus* in co-cultures. Recently, Schlegel et al. (2016) showed that ash leaf endophytes inhibited germination of *H. fraxineus* ascospores.

Although there have been many studies of tree endophytes, their diversity and spatio-temporal dynamics are still mostly unknown, which impedes the ability to utilize endophytes in forest protection (Louda et al. 2003, Jumpponen and Jones 2010, Witzell et al. 2014). Seasonal changes have been observed in the fungal community of the phyllosphere of deciduous trees (e. g. Sieber and Hugentobler 1987, Unterseher et al. 2007, Jumpponen and Jones 2010). However, less is known about the winter fungal community of coniferous and evergreen plants, as well as of bark of deciduous trees in temperate zones (Widler and Müller 1984, Buck et al. 1998, Osono 2008, Guo et al. 2008, Joshee et al. 2009). Although high-throughput sequencing methods may reveal total fungal communities, the origin of fungi (endophytic vs. epiphytic) may be difficult to assess (Jumpponen and Jones 2010). An advantage of cultivation methods is the yield of fungal isolates that can be further used for polyphasic identification and possible other applications such as antagonistic assays (Prior et al. 2014).

The aim of our study was to investigate the differences between fungal communities of healthy shoots of trees severely damaged by ash dieback and of those of relatively resistant trees. The comparison was made in two different seasons (summer and winter) which differ not only in climatic conditions but also in activity of the pathogen. A further objective was to assess the competitive ability of fungal endophytes naturally occurring in *F. excelsior* against *H. fraxineus*. For this purpose, isolated endophytes were tested in dual cultures *in vitro* on a nutrient-poor medium.

Materials and methods

Locations and collection of material

Field sampling was performed in July 2012 and February 2013 at two locations in the Czech Republic: Luž (50.8391469N, 14.6502289E, Lusatian mountains, 650 m a.s.l., mean annual temperature 6-7°C, mean annual precipitation 800-1000 mm) and Heřmanice (50.6555558N, 14.3852781E, Central Bohemian Uplands, 422 m a.s.l., mean annual temperature 7-8°C, mean precipitation 600-650 mm). Approximate meteorological data were obtained from the Climate Atlas of Czechia (Tolasz et al. 2007). Both localities had been exposed to infection pressure of *H. fraxineus* during previous years and trees showed various levels of ash dieback. The two locations consisted of windbreaks of adult ash trees along meadows and pastures, respectively. Three pairs of *F. excelsior* trees were chosen from each location. The distance between trees in a pair was 4 to 12 m to ensure similar conditions in the pair (growth conditions, sources of fungal inoculum, etc.). One tree from each pair visibly suffered from ash dieback (crown defoliation 30-50%) and the other was relatively resistant (crown defoliation up to 10%). Eight healthy looking one-year shoots were cut off from each tree at a height of approximately six metres and processed the next day.

Isolation and identification of fungal endophytes

In the laboratory, shoots were washed in tap water, divided into bark and wood and cut into fragments of 5×3-4 mm. Bark fragments were surface sterilized in 96% ethanol for 30 s, 0.47% sodium hypochlorite for 60 s, 96% ethanol for 30 s and finally rinsed in distilled water. The time of sterilization was shortened to 10 s in each disinfection agent for wood fragments. Five pieces of wood and five pieces of bark from one shoot were placed in 9 cm Petri dishes with 2% wort agar (2WA) prepared from brewery wort (Staropramen Brewery, Prague, Czech Republic). The final sucrose content was adjusted to 2% w/v, and the suspension was supplied with 18 g L-1 agar (Fassatiová 1986). Petri dishes were incubated in the dark at 20°C and checked weekly for the growth of fungi for four weeks for the summer sampling and six weeks for the winter sampling (due to almost no growth of fungi in the first two weeks). Emerging colonies were grouped into morphospecies based on phenotype characteristics and one isolate of each morphospecies was subcultured on 2WA for subsequent morphological identification and extraction of DNA. Molecular data were obtained using standard procedures for DNA extraction, PCR amplification with primers amplifying the ITS1-5.8S-ITS2 rDNA region and sequencing (Haňáčková et al. 2015). The morphological and molecular data were taken into consideration in the final identification of taxa and a currently accepted name was assigned to a taxon according to the Index Fungorum (http://www.indexfungorum.org/names/names.asp) or another relevant taxonomic source (e.g. recent monographies).

Antagonistic assays in dual cultures

Forty-nine isolates of fungal endophytes were tested against two isolates of *H. fraxineus*; these isolates of *H. fraxineus* were obtained from infected shoots of trees in the same locations (LUZ from Luž and HER from Heřmanice). All endophytes originated from *F. excelsior* except for *Biatriospora* sp. (strain CCF4378, obtained from the Culture Collection of Fungi, Charles University, Faculty of Science, Prague, Czech Republic), which was isolated from *Ulmus glabra* and was also recently detected in *Acer pseudoplatanus* (Kelnarová I., unpublished results). This isolate was considered to be a promising agent due to its broad spectrum of toxic secondary metabolites (Stodůlková et al. 2015). To approach natural conditions in our assays, agar with the addition of ash shoot extract and microcrystalline cellulose as a source of carbon was used in dual cultures. The extract was prepared as follows: *F. excelsior* shoots were cut into small fragments, boiled in water (30 g of fragments per 1 l of deionized water) for 20 min and filtered through a fine cloth. Agar medium was prepared from 1 l of the filtered suspension amended with 18 g of agar and 20 g of microcrystalline cellulose (Serva, Germany). Because of the slow growth of *H. fraxineus*, the pathogen was inoculated onto the Petri dish one week before an endophyte. Agar plugs with mycelium (5 mm diameter, taken from the actively growing margin of the colony) were placed 4 cm apart from each other on a 9 cm Petri dish. For the self-inhibition test, the identical *H. fraxineus* isolate was used instead of an endophyte. The negative control consisted of pairing *H. fraxineus* with a plug of agar. Each treatment was replicated three times and the growth of the pathogen was measured as a colony radius on a connective line between both colonies and on a line 45° up and down from this line after 14 and 28 days of co-cultivation at 18°C. At the end of cultivation, five plugs from the interaction zone or from the margin of the *H. fraxineus* colony (in the case of no contact) were transferred to 2WA to reisolate the pathogen to confirm viability of *H. fraxineus* in the contact zone and distinguish between replacement and overgrowing only (Koukol et al. 2006).

Data analysis

Isolated endophytes were classified into three presumptive groups: pathogens, saprotrophs, or endophytes with unknown ecology, according to available records about given species or higher taxa in the literature or GenBank (http://www.ncbi.nlm.nih.gov). The colonization frequency of each taxon was calculated as the proportion of colonies from the total number of colonies per sample and the Shannon index was defined for fungal communities. Shannon indices were compared using paired *t*-tests. The dependency of the occurrence of particular fungal species on season and the health status of the tree was assessed using contingency table chi-squared tests.

To compare the inhibition ability of endophytes, data for both isolates of *H. fraxineus* were assessed together due to similar variances. The sum of *H. fraxineus* growth in three directions after 28 days was converted to a relative scale (1 = maximal growth of *H. fraxineus* without other fungus, 0 = no growth of *H. fraxineus*) and was further explained as an inhibition effect of an endophyte. Differences in the inhibition effects of endophytes were assessed using two-way ANOVA. Homogeneous groups of endophytes (inhibition effect of endophytes in the same group was not significantly different) were defined using Tukey multiple comparisons of means. The statistical analyses were performed in PAST: Paleontological Statistics Software Package for Education and Data Analysis (Hammer et al. 2001) and Statistica 7.0 (StatSoft, Inc., Tulsa, OK).

Results

Endophytic community

In total, the isolation yielded 884 colonies representing 58 fungal species; of these species, 35 were obtained in the summer and 38 in the winter (Table 1). Twenty species were isolated only in the summer, 23 only in winter and 15 were present in both seasons. Shannon diversity was significantly higher in the winter ($P < 0.001$). The number of fungal colonies also increased from 382 in summer to 502 in winter. Irrespective of the health status of the tree, the species richness of saprotrophs decreased and the species richness of pathogens increased in the winter (Table 2). The number of species was higher in the shoots of resistant trees than in susceptible trees (32 and 26, respectively), but the number of colonies was identical (442 in each).

The most frequent taxa in summer were unidentified yeast (21%), *Aureobasidium pullulans* (19%) and *Phoma macrostoma var. incolorata* (10%), whereas Dothideomycetes sp. 1 (20%), *Diaporthe* sp. 2 (13%) and *Lophiostoma* sp. (12%) dominated in the winter. Seasonal changes in frequencies were significant for 23 species (Table 1). Seasonality was apparent for several frequent species, e. g. *A. pullulans*, Dothideomycetes sp. 1, *Diaporthe* sp. 2, both varieties of *P. macrostoma*, both species of *Lophiostoma* or Pleosporales sp. 3. In the winter, *Diaporthe* sp. 2 was significantly associated with susceptible trees ($P = 0.02$). Similarly, *Diaporthe* sp. 1 reached higher numbers in susceptible trees in the summer, but this finding was not statistically significant ($P = 0.095$). Nevertheless, total communities did not significantly differ between susceptible and resistant trees.

The growth of H. fraxineus in dual cultures

The inhibition of *H. fraxineus* was significantly affected by the endophyte, by the used isolate of *H. fraxineus* and by their interaction in dual cultures after 28 days (Table 3). When the results from both isolates were combined, 36 endophytes had a significantly inhibiting effect on the growth of *H. fraxineus*. The inhibitions varied from 42 to 83%. The best inhibition rates, over 80%, were achieved by *Botrytis cinerea* and *Phoma macrostoma* var. *incolorata* (Figure 1). Another 26 species caused growth inhibition of *H. fraxineus* by at least 50%. In contrast, 11 species had lower antagonistic effects than *H. fraxineus* itself, i.e. less than 39% inhibition, but these species belonged to one homogeneous group with self-inhibition of *H. fraxineus*. When inhibition rates were compared with self-inhibition of *H. fraxineus*, only 17 endophytes had significantly higher effect than self-inhibition. No reisolation of *H. fraxineus* was yielded after interaction with three endophytes: *Gibberella baccata*, *Lopadostoma turgidum* and *Nemania serpens* suggesting replacement of *H. fraxineus* by these endophytes. However, morphological changes on mycelium were not observed. Dual cultures with some species with good inhibition rates, e.g., *B. cinerea* and *Alternaria alternata*, also resulted in poor reisolation (one positive out of five) of the pathogen. However, the results of many other endophytes differed for the two *H. fraxineus* isolates. The LUZ isolate was not reisolated after interaction with 14 endophytes, whereas the HER isolate was not reisolated after interaction with three endophytes. *Biatriospora* sp. affected *H. fraxineus* with 40% inhibition and reisolation of the pathogen was successful (three positive out of five). The inhibition rates of endophytes (for each isolate of *H. fraxineus*), the results of reisolation and identification of endophyte species are available in the Supplementary Data (Suppl. Table 1 and Suppl. Table 2).

Discussion

To be complementary to previous studies aimed at (micro)fungi colonizing ash twigs and shoots, we combined data characterising the composition of the fungal community from two different seasons and two types of trees differing in health condition with the antagonistic potential of isolated fungal species against *H. fraxineus*. Using this approach, we were able to obtain a complex view of the function of endophytic mycobiota against the spread of *H. fraxineus* in shoot tissue.

The fungal community and seasonal changes

We recorded similar magnitudes of the overall species number as in previous studies using the same substrate, considering some variation in the sampling design and isolation methods. However, the dominant species differed substantially. *Aureobasidium pullulans*, one of the most frequently isolated species in our study and in the study by Davydenko et al. (2013), was recorded with medium frequency by Bakys et al. (2009b) and only rarely by Kowalski and Kehr (1992) (Table 4). Some taxa were congruent with endophytes from other ash tissues. An unknown member of Pleosporales (Pleosporales sp. 1) was isolated from ash shoots with advanced necrosis and also from living leaves (identical to Fungal sp. 104 in Bakys et al. 2009a and to Fungal sp. MT0843 in Scholtysik et al. 2013, respectively). *Coniothyrium* sp. was recorded in necrotic ash leaves and shoot bark (Bakys et al. 2009b). *Dendrothyrium* sp., *Bjerkandera adusta* and *Coprinellus disseminatus* were recorded in ash shoots with dead tops (Bakys et al. 2009a).

Table 1. Frequencies of isolation of fungi (%) in summer and winter sampling for all samples and for susceptible/resistant trees. When known, the ecological role is provided for each taxon: p = pathogen, s = saprotroph, ps = both strategies, ? = unknown ecology

Order	Fungal taxa	Ecology	Summer (all)	Summer (susceptible / resistant)	Winter (all)	Winter (susceptible / resistant)
Pleosporales	*Alternaria alternata*	ps	1.83	(1.52|2.16)	1.99	(2.04|1.95)
	Coniothyrium sp.*	ps	–	(-|-)	0.8	(1.63|-)
	Dendrothyrium sp.	p	–	(-|-)	1.39	(-|2.72)
	*Lophiostoma corticola**	s	1.31	(2.03|0.54)	6.18	(6.12|6.23)
	Lophiostoma sp.*	s	–	(-|-)	12.35	(15.5|9.34)
	*Phoma macrostoma**	ps	4.97	(5.08|4.86)	–	(-|-)
	Phoma macrostoma var. *incolorata**	ps	10.21	(8.12|12.43)	2.79	(2.86|2.72)
	Phoma sp.	s	0.79	(0.51|1.08)	1.59	(-|3.11)
	Pleospora herbarum	ps	0.26	(-|0.54)	–	(-|-)
	Pleosporales sp. 1*	?	4.45	(2.54|6.49)	0.4	(-|0.78)
	Pleosporales sp. 2	s	0.79	(0.51|1.08)	–	(-|-)
	Pleosporales sp. 3*	?	–	(-|-)	8.76	(6.94|10.5)
	Pyrenochaeta corni	ps	1.83	(1.02|2.7)	1.39	(0.82|1.95)
Dothideales	*Aureobasidium pullulans**	s	19.11	(20.81|17.3)	5.98	(4.9|7)
Capnodiales	*Cladosporium* sp.*	?	2.88	(2.54|3.24)	–	(-|-)
incertae sedis	Dothideomycetes sp. 1*	?	0.79	(1.02|0.54)	20.32	(22.0|18.6)
	Dothideomycetes sp. 2	?	0.52	(1.02|-)	–	(-|-)
	Dothideomycetes sp. 3	s	–	(-|-)	0.2	(0.41|-)
	Dothideomycetes sp. 4	?	–	(-|-)	0.2	(-|0.39)
	All Dothideomycetes		49.74	(46.72|52.96)	64.34	(63.27|65.38)
Xylariales	*Annulohypoxylon cohaerens*	s	0.26	(0.51|-)	0.2	(-|0.39)
	Annulohypoxylon multiforme	s	0.26	(0.51|-)	1	(0.41|1.56)
	Anthostomella pinea	ps	2.88	(1.02|4.86)	1.99	(1.63|2.33)
	Lopadostoma turgidum	s	–	(-|-)	0.2	(0.41|-)
	Nemania serpens	s	0.26	(0.51|-)	–	(-|-)
	Xylaria longipes	s	0.26	(0.51|-)	–	(-|-)
	Xylariales sp. 1*	?	–	(-|-)	0.8	(0.82|0.78)
	Xylariales sp. 2	?	–	(-|-)	0.2	(0.41|-)
Diaporthales	*Apiognomonia errabunda*	p	–	(-|-)	0.2	(-|0.39)
	Cryptodiaporthe hystrix	ps	–	(-|-)	0.6	(0.82|0.39)
	Diaporthe sp. 1*	?	4.97	(6.6|3.24)	–	(-|-)
	Diaporthe sp. 2*	?	–	(-|-)	13.35	(17.5|9.34)
	*Prosthecium platanoidis**	p	–	(-|-)	2.79	(2.04|3.5)
	Prosthecium pyriforme	p	0.26	(0.51|-)	–	(-|-)
	Valsa sp.*	p	1.5	(0.51|1.62)	–	(-|-)
Hypocreales	*Gibberella avenacea*	ps	1.83	(2.54|1.08)	1	(-|1.95)
	*Gibberella baccata**	ps	3.14	(3.05|3.24)	0.4	(-|0.78)
Sordariales	*Chaetomium globosum**	s	1.5	(1.52|0.54)	–	(-|-)
	Sordaria fimicola	s	0.79	(1.02|0.54)	–	(-|-)
Coniochaetales	*Coniochaeta* sp.	ps	0.26	(-|0.54)	–	(-|-)
incertae sedis	Sordariomycetes sp. 1	?	0.26	(-|0.54)	–	(-|-)
	Sordariomycetes sp. 2	?	–	(-|-)	1	(0.41|1.56)
	Sordariomycetes sp. 3	?	–	(-|-)	0.4	(0.82|-)
	All Sordariomycetes		17.54	(18.81|16.2)	24.1	(25.32|22.97)

Table 1. (Continued)

Order	Fungal taxa	Ecology	Summer (all)	Summer (susceptible / resistant)	Winter (all)	Winter (susceptible / resistant)
Helotiales	*Botrytis cinerea*	ps	0.52	(0.51|0.54)	–	(-|-)
	Bulgaria inquinans	s	0.79	(1.02|0.54)	–	(-|-)
	Helotiales sp.*	s	–	(-|-)	4.58	(4.49|4.67)
	Pezicula sporulosa	p	–	(-|-)	1	(2.04|-)
	Phialocephala sp.	s	–	(-|-)	0.4	(0.41|0.39)
incertae sedis	Leotiomycetes sp. 1*	?	–	(-|-)	1	(1.22|0.78)
	Leotiomycetes sp. 2	?	–	(-|-)	0.2	(-|0.39)
	All Leotiomycetes		1.31	(1.53|1.08)	7.17	(8.16|6.23)
Eurotiales	*Aspergillus pseudoglaucus*	s	1.57	(2.03|1.08)	–	(-|-)
	Aspergillus versicolor	s	0.52	(-|1.08)	0.2	(-|0.39)
	*Penicillium citrinum**	s	2.88	(4.06|1.62)	–	(-|-)
Chaetothyriales	*Phaeomoniella* sp.*	p	–	(-|-)	2.79	(2.04|3.5)
	All Eurotiomycetes		4.97	(6.09|3.78)	2.99	(2.04|3.89)
Polyporales	*Bjerkandera adusta*	s	–	(-|-)	0.2	(-|0.39)
Agaricales	*Coprinellus disseminatus*	s	–	(-|-)	0.2	(-|0.39)
	All Agaricomycetes		–	(-|-)	0.4	(-|0.78)
Sporidiobolales	*Rhodotorula mucilaginosa**	s	4.97	(7.11|2.7)	–	(-|-)
	All Microbotryomycetes		4.97	(7.11|2.7)	–	(-|-)
Pezizales	*Desmazierella acicola*	s	0.26	(-|0.54)	–	(-|-)
	All Pezizomycetes		0.26	(-|0.54)	–	(-|-)
incertae sedis	unidentified yeast*	?	21.2	(19.8|22.7)	1	(1.22|0.78)
	All *incertae sedis*		21.2	(19.8|22.7)	1	(1.22|0.78)

* indicates a significant difference in the frequency of the taxa between the seasons ($P < 0.05$)

Table 2. Species richness and number of fungal colonies isolated from healthy ash shoots as a function of season and with attributed ecological group. Isolates with unknown ecology were not included

Ecological group of fungi	Summer		Winter	
	No. of colonies	Species richness	No. of colonies	Species richness
Saprotrophs	149	16	167	13
Pathogens	5	2	41	7
Both strategies	105	10	55	8

Pyrenochaeta corni was noted as an endophyte of living ash leaves (Scholtysik et al. 2013) and current year seeds (Cleary et al. 2013). Our approach most likely excluded some yeasts that are typical for the bark and wood of *F. excelsior* (Bakys et al. 2009b, Chen 2012). We isolated just two yeasts: *Rhodotorula mucilaginosa* and an unidentified yeast.

Several species that we recorded as singletons were never before recorded in any type of ash tissue. For example, we recorded *Desmazierella acicola*, a saprotroph of pine needles (Martinović et al. 2016) and *Prosthecium platanoides*, which was previously only known to be restricted to dead branches of *Acer pseudoplatanus* (Voglmayr and Jaklitsch 2008). Similar findings were noted by Hayatgheibi (2013), who isolated *Lophodermium pinastri*, an endophyte and later saprotroph of pine needles, from ash seeds. These findings may represent accidental infections from surrounding vegetation (Kowalski and Kehr 1992, Joshee et al. 2009), and suggest interesting host flexibility, but probably without any significance for a given fungal species and impact on the host.

We revealed a clear seasonal change in the fungal community of ash shoots with a higher species richness of pathogenic fungi in the winter (Table 2). The dormant season of trees probably facilitates the growth of pathogens,

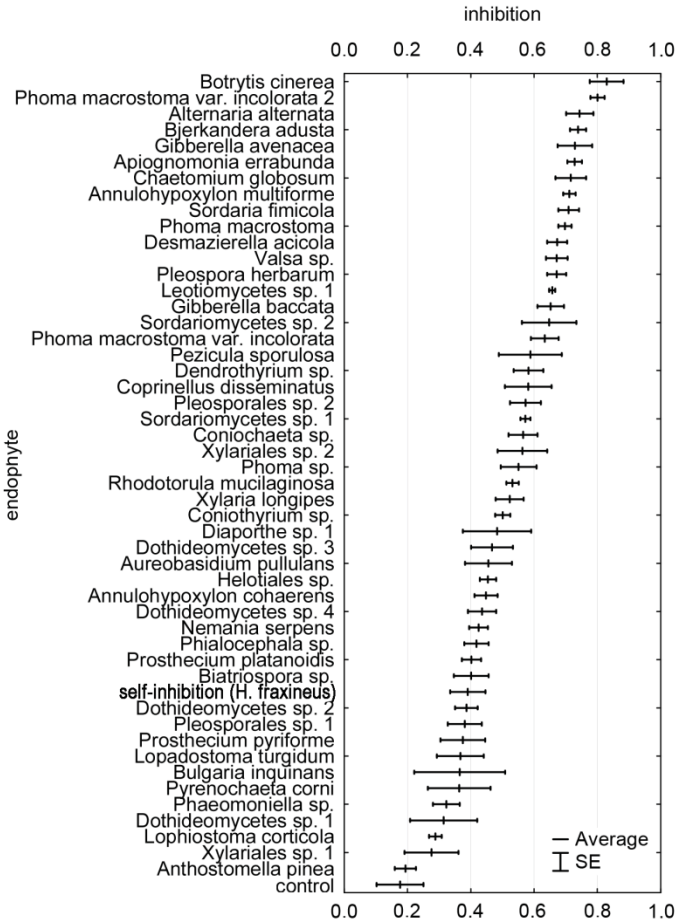

Figure 1. Average growth inhibition of *H. fraxineus* caused by endophytes isolated from healthy ash shoots (including *Biatriospora* sp. from CCF). Horizontal bars indicate standard errors. Identical isolates of *H. fraxineus* were used to test the self-inhibition and a pairing with an agar plug was used in the control treatment

Table 3. Results of the two-way ANOVA of *H. fraxineus* growth inhibition by endophytes in dual culture. (*Df* = degrees of freedom, Sum *Sq* = sum of squares, Mean *Sq* = mean squares, E = endophyte, I = isolate of *H. fraxineus*)

Source of variation	*Df*	Sum *Sq*	Mean *Sq*	*F* value	Pr(> F)
Endophyte (E)	50	7.668	0.15336	15.421	<2e-16 ***
H. fraxineus (I)	1	0.061	0.06104	6.138	0.0141 *
E*I	50	2.694	0.05389	5.419	<2e-16 ***
Residuals	199	1.979	0.00994		

especially when winters are mild (Lonsdale and Gibbs 1996). Moreover, the decrease in carbohydrate content in twigs during the winter and the translocation of carbohydrates to storage tissues – frequently roots (Kozlowski et al. 1991) – can reduce the survival or activity of saprotrophs in shoot tissues. Winter shoots were older than summer shoots, which could also increase the number of species, which is known to be somewhat positively correlated with the age of tissue (Widler and Müller 1984, Guo et al. 2008).

The fungal community of trees differing in health status

The influence of tree health status on fungal endophytes was studied in healthy shoots of six pairs of *F. excelsior*. Susceptible ash trees hosted more colonies of *Diaporthe* sp. 1 and *Diaporthe* sp. 2. *Diaporthe* species (with anamorphs assigned to *Phomopsis*) have often been isolated as endophytes (Sieber 2007) and frequently reported as plant

Table 4. A comparison of endophytic fungi detected in healthy ash (*F. excelsior*) shoots from different countries

	Bakys et al. 2009a	Chen 2012*	Davydenko et al. 2013	Kowalski and Kehr 1992*	present study	
Country of origin	Sweden	New Zealand	Ukraine	Germany, Poland	Czech Republic	
Sampling season (month)	summer (June)	winter (August)	spring (May)	thourough summer and autumn	summer (July)	winter (February)
Number of samples	58 (wood with bark)	34 bark + 35 wood	68 (wood with bark)	70 (wood with bark)	92 (wood with bark)	92 (wood with bark)
Isolation of endophytes	cultivation (Hagem agar)	cultivation (1%MEA) and direct DNA extraction with cloning	direct DNA extraction and sequencing of fungal ITS rRNA	cultivation (2% MEA)	cultivation (2% wort agar)	
Number of species	20	16 and 53	7	47	35	38
Dominant species	*Alternaria alternata* *Epicocum nigrum* *Giberella avenacea*	Ascomycota sp. *Fusarium* sp. unidentified sp. 119	*Alternaria arborescens* *Aureobasidium pullulans* *Cladosporium cladosporioides* *Cladosporium tenuissimum*	*Alternaria alternata* *Pezicula cinnamomea* *Phomopsis* spp.	*Aureobasidium pullulans* *Phoma macrostoma* var. *incolorata* unidentified yeast	*Diaporthe* sp. 2 Dothideomycetes sp. 1 *Lophiostoma* sp.
Species congruently recorded in present study	*Alternaria alternata* *Aureobasidium pullulans* *Diaporthe* sp. 1 *Gibberella avenacea* *Valsa* sp.	*Gibberella baccata* *Lophiostoma corticola*	*Aureobasidium pullulans*	*Aureobasidium pullulans* *Alternaria alternata* *Prosthecium pyriforme* *Nemania serpens*		

*only endophytes identified to the species level were compared due to a lack of accession numbers

pathogens (Tan et al. 2013). Trees suffering from ash dieback thus probably enable wider expansion of other parasites in plant tissue as observed on *Phomopsis oblonga* in trees affected by *Ophiostoma* spp. (Webber and Gibbs 1984). Additionally, reduced defensive capacity of a tree can trigger a shift of an endophyte to a pathogenic phase (Sieber 2007). However, it is difficult to differentiate whether there are beneficial effects of *H. fraxineus* on the development of other pathogens or if tissues previously colonized by other pathogens accelerate the spread of *H. fraxineus* and progress of the ash dieback.

Sieber and Hugentobler (1987) focused on endophytic assemblages in healthy leaves and twigs of healthy and diseased beech trees (*Fagus sylvatica*). Similarly to our results, they did not find differences between endophytic communities from those substrates. In contrast to our results, *Diaporthe eres* had higher frequencies in the leaves of healthy trees, which was attributed to supposedly higher water capacity compared to diseased trees. Similarly, Gennaro et al. (2003) did not demonstrate a distinction between whole endophytic communities of healthy and declining *Quercus* species. However, these authors noted a significantly lower Shannon-Wiener index for leaves, twigs and buds of declining *Q. robur* and shoots of declining *Q. cerris*. Although trees in our study did not differ significantly in species diversity, we isolated more species from resistant trees. Ragazzi et al. (2003) found significantly higher colonization frequencies of given fungal species in twigs and symptomless leaves of declining *Quercus* species in comparison to healthy ones. These authors emphasized that this pattern was especially evident for fungal species that can switch to a pathogenic lifestyle. It seems that trees in a better state of health can host

more diversified fungal communities, or higher species diversity of fungi balances the self-assertion of opportunistic pathogens. A tree probably chooses from several methods of protecting itself against fungal pathogens. A high production of phenolic compounds can also limit fungal spread, and in that case, low species diversity of endophytes is a sign of resistance. This feature of a tree can even be restricted to a particular type of tissue (Martín et al. 2013). The end of active restriction of the endophytic fungal community may be recognized after tissue dieback, when a shoot becomes accessible for various pathogens and saprotrophs. At a certain moment of infection, species richness of fungal taxa in necrotic shoots significantly increases (Bakys et al. 2009a, Davydenko et al. 2013).

The influence of ash endophytes on H. fraxineus growth in vitro

Here, we examined the inhibition effect of native endophytes colonizing European *F. excelsior* in interactions with non-native pathogenic *H. fraxineus*. Medium with ash extract was used to simulate natural conditions for interactions because this antagonistic screening will be followed by *in planta* tests in ash seedlings. Different media and cultivation conditions can influence the results of antagonism substantially (Kusari et al. 2013). The use of media rich in nutrients such as MEA or PDA could support isolates in faster growth or production of secondary metabolites that cannot synthesize in plant tissue as endophytes. There are also obvious limits of the pairwise testing, which ignores the collective effect of endophytes. This study tried to overcome this bias by using a simultaneous view of the mosaic of endophytes present in ash shoots. The majority of the endophytes used in our study inhibited the growth of both tested *H. fraxinus* isolates. Nevertheless, inhibition rates of more than half endophytes were comparable with self-inhibition of *H. fraxineus*. Similarly, Schulz et al. (2015) reported that 57 of 59 tested endophytes inhibited *H. fraxineus*. However, only 19 of those 59 reached inhibition rates greater than 30%.

The endophytes with the best inhibition rates were mostly fast growing species and some of them, such as *P. macrostoma* var. *incolorata*, *G. baccata* or *A. pullulans*, reached high frequencies in shoots in summer. The synergistic effect of these endophytes could reduce the number of *H. fraxineus* strains infecting shoots in the late summer or autumn. The lower frequencies of these endophytes in the winter could contribute to the spread of *H. fraxineus*. However, *P. macrostoma* (both varieties) and *G. baccata* are weak parasites (De Gruyter et al. 2002, Leslie and Summerell 2006). These species were previously isolated from ash shoots with necrosis and might not cause disease, but might profit from *H. fraxineus* infection (Przybył 2002, Bakys et al. 2009a). Not all genotypes of a particular species are able to be virulent to a particular host. Long co-evolution of the endophyte with the host tree is assumed to result in lower susceptibility of the tree. High colonization frequencies of an endophyte are even considered to be a signal of low virulence (Sieber 2007). Surprisingly, *Biatriospora* sp., known to produce a mixture of antagonistic compounds (Stodůlková et al. 2015), had only intermediate effect on the pathogen.

Although we did not establish controls for endophytes, reduction in their growth was often apparent, likely as a result of reciprocal antagonism, which is assumed for most of the endophytic microbes. Antagonism among different isolates of the same species is no exception (Schulz et al. 2015, Yan et al. 2015). The impact of *H. fraxineus* on the native community of endophytes was recently outlined by Schulz et al. (2015), who reported that 13 of 55 tested ash endophytes were inhibited by more than 30% by *H. fraxineus*.

Conclusion

The endophytic fungal community of selected ash shoots did not differ between trees susceptible to ash dieback and resistant trees. This finding could indicate that the endophyte community has a lower impact on the inhibition of *H. fraxineus*, and that trees with better health status defend themselves by other mechanisms. Although dual tests with ash endophytes demonstrated their significant influence on *H. fraxineus* growth and suggested which should be tested in future *in vivo* trials, reciprocal antagonism was observed as well; thus, many species do not represent an obstacle for *H. fraxineus*, suggesting that a neutral outcome of the interaction can occur *in vivo*. In contrast, a decrease in some fast-growing species in the winter could facilitate the growth of *H. fraxineus* in colder months. Reduced numbers of tree saprotrophs in the winter also might be relevant for the success of other pathogenic fungi.

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Supplementary material

Suppl. Table 1. Inhibition rates of endophytes and the results of *H. fraxineus* reisolation after interaction

Suppl. Table 1a. Average inhibition rate of the endophytes againts two isolates of *H. fraxineus*. Endophytes connected with the same vertical line belong to the same homogeneous group and are not significantly different ($P > 0.05$) according to Tukey multiple comparisons of means. Identical isolates of *H. fraxineus* were used to test the self-inhibition and a pairing with an agar plug was used in the control treatment

Endophyte	Inhibition rate	Homogeneous groups
Botrytis cinerea	0.83		
Phoma macrostoma var. *incolorata 2*	0.8			
Bjerkandera adusta	0.74						
Alternaria alternata	0.74					
Apiognomonia errabunda	0.73						
Gibberella avenacea	0.73					
Chaetomium globosum	0.72						
Sordaria fimicola	0.71						
Annulohypoxylon multiforme	0.71						
Phoma macrostoma	0.7							
Pleospora herbarum	0.67								
Valsa sp.	0.67								
Desmazierella acicola	0.67								
Leotiomycetes sp. 1	0.66									
Sordariomycetes sp. 2	0.65										
Gibberella baccata	0.65									
Phoma macrostoma var. *incolorata*	0.63											
Pezicula sporulosa	0.59											
Coprinellus disseminatus	0.58											
Dendrothyrium sp.	0.58											
Coniochaeta sp.	0.57												
Sordariomycetes sp. 1	0.57											
Pleosporales sp. 2	0.57											
Xylariales sp. 2	0.56											
Phoma sp.	0.55												
Rhodotorula mucilaginosa	0.53												

Suppl. Table 1.a (Continued)

Endophyte	Inhibition rate	Homogeneous groups
Xylaria longipes	0.52	| | | | | | | | | | |
Coniothyrium sp.	0.5	| | | | | | | | | | | |
Diaporthe sp. 1	0.48	| | | | | | | | | | |
Dothideomycetes sp. 3	0.47	| | | | | | | | |
Aureobasidium pullulans	0.46	| | | | | | | |
Annulohypoxylon cohaerens	0.45	| | | | | | | |
Helotiales sp.	0.45	| | | | | | | |
Dothideomycetes sp. 4	0.44	| | | | | | | |
Phialocephala sp.	0.42	| | | | | | | |
Nemania serpens	0.42	| | | | | | | |
Biatriospora sp.	0.4	| | | | | | |
Prosthecium platanoidis	0.4	| | | | | | |
Dothideomycetes sp. 2	0.39	| | | | | |
self-inhibition *(H. fraxineus)*	0.39	| | | | | |
Prosthecium pyriforme	0.38	| | | | | |
Pleosporales sp. 1	0.38	| | | | | |
Bulgaria inquinans	0.37	| | | | | |
Lopadostoma turgidum	0.37	| | | | | |
Pyrenochaeta corni	0.36	| | | | | |
Phaeomoniella sp.	0.32	| | | | |
Dothideomycetes sp. 1	0.31	| | | | |
Lophiostoma corticola	0.29	| | | |
Xylariales sp. 1	0.28	| | | |
Anthostomella pinea	0.19	| | |
control	0.18	|

Suppl. Table 1b. Average inhibition rate of the endophytes againts *H. fraxineus* isolate LUZ. Endophytes connected with the same vertical line belong to the same homogeneous group and are not significantly different ($P > 0.05$) according to Tukey multiple comparisons of means. Identical isolates of *H. fraxineus* were used to test the self-inhibition and a pairing with an agar plug was used in the control treatment.

Endophyte	Inhibition rate	Homogeneous groups
Botrytis cinerea	0.92	|
Phoma macrostoma var. *incolorata* 2	0.84	| |
Alternaria alternata	0.84	| |
Chaetomium globosum	0.78	| | |
Pezicula sporulosa	0.76	| | |
Desmazierella acicola	0.73	| | | |
Apiognomonia errabunda	0.72	| | | | | |
Phoma macrostoma	0.72	| | | | | |
Coprinellus disseminatus	0.72	| | | | |
Annulohypoxylon multiforme	0.71	| | | | | |
Gibberella avenacea	0.7	| | | | | | |
Bjerkandera adusta	0.68	| | | | | | | | | | | |
Leotiomycetes sp. 1	0.67	| | | | | | | |
Pleospora herbarum	0.66	| | | | | | | |
Sordaria fimicola	0.65	| | | | | | | | |
Valsa sp.	0.63	| | | | | | | | | |
Gibberella baccata	0.63	| | | | | | | | | |
Phoma macrostoma var. *incolorata*	0.61	| | | | | | | | | | |

Suppl. Table 1.b (Continued)

Endophyte	Inhibition rate	Homogeneous groups
Sordariomycetes sp. 1	0.59	| | | | | | | | | | | |
Pleosporales sp. 2	0.59	| | | | | | | | | | | |
Dendrothyrium sp.	0.57	| | | | | | | | | | | |
Dothideomycetes sp. 3	0.57	| | | | | | | | | | | |
Coniothyrium sp.	0.54	| | | | | | | | | | | |
Rhodotorula mucilaginosa	0.53	| | | | | | | | | | | |
Phoma sp.	0.5	| | | | | | | | | | | | |
self-inhibition (*H. fraxineus*)	0.5	| | | | | | | | | | | | |
Coniochaeta sp.	0.49	| | | | | | | | | | | | |
Aureobasidium pullulans	0.49	| | | | | | | | | | | | |
Sordariomycetes sp. 2	0.49	| | | | | | | | | | | | |
Xylaria longipes	0.48	| | | | | | | | | | | | |
Pleosporales sp. 1	0.46	| | | | | | | | | | | | | |
Helotiales sp.	0.45	| | | | | | | | | | | | | |
Phialocephala sp.	0.41	| | | | | | | | | | | | | |
Xylariales sp. 2	0.4	| | | | | | | | | | | | | |
Nemania serpens	0.39	| | | | | | | | | | | | | |
Prosthecium platanoidis	0.39	| | | | | | | | | | | | | |
Annulohypoxylon cohaerens	0.38	| | | | | | | | | | | | | |
Xylariales sp. 1	0.37	| | | | | | | | | | | | | |
Dothideomycetes sp. 4	0.35	| | | | | | | | | | | | | |
Dothideomycetes sp. 2	0.34	| | | | | | | | | | | | | |
control	0.34	| | | | | | | | | | | | | |
Phaeomoniella sp.	0.34	| | | | | | | | | | | | | |
Lophiostoma corticola	0.32	| | | | | | | | | | | | | |
Biatriospora sp.	0.3	| | | | | | | | | | | | | |
Lopadostoma turgidum	0.28	| | | | | | | | | | | | | |
Anthostomella pinea	0.25	| | | | | | | | | | | | | |
Diaporthe sp. 1	0.24	| | | | | | | | | | | | | |
Prosthecium pyriforme	0.23	| | | | | | | | | | | | | |
Pyrenochaeta corni	0.17	| | | | | | | | | | | | | |
Bulgaria inquinans	0.13	| | | | | | | | | | | | | |
Dothideomycetes sp. 1	0.09	| | | | | | | | | | | | | |

Suppl. Table 1c. Average inhibition rate of the endophytes againts *H. fraxineus* isolate HER. Endophytes connected with the same vertical line belong to the same homogeneous group and are not significantly different ($P > 0.05$) according to Tukey multiple comparisons of means. Identical isolates of *H. fraxineus* were used to test the self-inhibition and a pairing with an agar plug was used in the control treatment

Endophyte	Inhibition rate	Homogeneous groups
Sordariomycetes sp. 2	0.8	|
Phoma macrostoma var. *incolorata* 2	0.77	| |
Sordaria fimicola	0.77	| |
Gibberella avenacea	0.76	| | |
Bjerkandera adusta	0.76	| | |
Apiognomonia errabunda	0.74	| | | |
Botrytis cinerea	0.74	| | | |
Xylariales sp. 2	0.73	| | | |
Bulgaria inquinans	0.71	| | | | |
Valsa sp.	0.71	| | | |

Suppl. Table 1.c (Continued)

Endophyte	Inhibition rate	Homogeneous groups
Annulohypoxylon multiforme	0.71	| | | |
Gibberella baccata	0.68	| | | | |
Coniochaeta sp.	0.68	| | | | | |
Phoma macrostoma	0.68	| | | | |
Pleospora herbarum	0.68	| | | | |
Chaetomium globosum	0.66	| | | | |
Phoma macrostoma var. *incolorata*	0.66	| | | | |
Leotiomycetes sp. 1	0.65	| | | | |
Alternaria alternata	0.65	| | | | |
Diaporthe sp. 1	0.64	| | | | |
Desmazierella acicola	0.61	| | | | | | |
Dendrothyrium sp.	0.6	| | | | | | | |
Phoma sp.	0.6	| | | | | | | |
Sordariomycetes sp. 1	0.56	| | | | | | | | |
Pyrenochaeta corni	0.56	| | | | | | | | |
Xylaria longipes	0.56	| | | | | | | | |
Pleosporales sp. 2	0.55	| | | | | | | | |
Dothideomycetes sp. 1	0.54	| | | | | | | | |
Rhodotorula mucilaginosa	0.53	| | | | | | | | |
Annulohypoxylon cohaerens	0.52	| | | | | | | | |
Dothideomycetes sp. 4	0.52	| | | | | | | | |
Prosthecium pyriforme	0.52	| | | | | | | | |
Biatriospora sp.	0.51	| | | | | | | | | |
Nemania serpens	0.46	| | | | | | | | | | |
Coniothyrium sp.	0.46	| | | | | | | | | | |
Helotiales sp.	0.46	| | | | | | | | | | |
Lopadostoma turgidum	0.45	| | | | | | | | | | |
Dothideomycetes sp. 2	0.44	| | | | | | | | | | |
Coprinellus disseminatus	0.44	| | | | | | | | | | |
Phialocephala sp.	0.43	| | | | | | | | | | |
Aureobasidium pullulans	0.42	| | | | | | | | | | |
Pezicula sporulosa	0.42	| | | | | | | | | | |
Prosthecium platanoidis	0.41	| | | | | | | | | | |
Dothideomycetes sp. 3	0.37	| | | | | | | | | | |
Pleosporales sp. 1	0.31	| | | | | | | | | | | |
Phaeomoniella sp.	0.3	| | | | | | | | | | | |
self-inhibition (*H. fraxineus*)	0.28	| | | | | | | | | | | |
Lophiostoma corticola	0.26	| | | | | | | | | | | |
Xylariales sp. 1	0.18	| | | | | | | | | | | |
Anthostomella pinea	0.13	| | | | | | | | | | | |
control	0.01	| | | | | | | | | | | |

Suppl. Table 1d. Average reisolation of both isolates of *H. fraxineus* from five discs cut from the interaction zone with the given endophyte species. The endophytes are ordered alphabetically

Endophyte species	Average reisolation		Endophyte species	Average reisolation	
	Isolate HER	Isolate LUZ		Isolate HER	Isolate LUZ
Alternaria alternata	2.0	0.0	*Lopadostoma turgidum*	0.3	0.0
Annulohypoxylon cohaerens	5.0	5.0	*Lophiostoma corticola*	4.3	0.0
Annulohypoxylon multiforme	5.0	5.0	*Nemania serpens*	0.0	0.0
Anthostomella pinea	5.0	5.0	*Pezicula sporulosa*	3.7	0.0
Apiognomonia errabunda	4.7	5.0	*Phaeomoniella* sp.	5.0	5.0
Aureobasidium pullulans	1.0	2.7	*Phialocephala* sp.	2.0	3.7
Biatriospora sp.	3.0	3.7	*Phoma macrostoma*	5.0	4.3
Bjerkandera adusta	3.0	0.0	*Phoma macrostoma* var. *incolorata*	4.7	0.0
Botrytis cinerea	1.3	0.0	*Phoma macrostoma* var. *incolorata* 2	5.0	0.0
Bulgaria inquinans	5.0	5.0	*Phoma* sp.	0.3	1.0
Coniochaeta sp.	5.0	3.7	*Pleospora herbarum*	3.7	1.3
Coniothyrium sp.	5.0	0.0	Pleosporales sp. 1	3.3	5.0
Coprinellus disseminatus	3.7	2.7	Pleosporales sp. 2	4.7	3.3
Dendrothyrium sp.	3.7	3.0	*Prosthecium platanoidis*	5.0	3.3
Desmazierella acicola	1.7	2.7	*Prosthecium pyriforme*	5.0	5.0
Diaporthe sp. 1	1.3	2.3	*Pyrenochaeta corni*	5.0	1.7
Dothideomycetes sp. 1	4.3	5.0	*Rhodotorula mucilaginosa*	4.7	5.0
Dothideomycetes sp. 2	3.0	1.7	*Sordaria fimicola*	5.0	5.0
Dothideomycetes sp. 3	1.7	0.0	Sordariomycetes sp. 1	1.0	5.0
Dothideomycetes sp. 4	5.0	5.0	Sordariomycetes sp. 2	4.0	0.0
Gibberella avenacea	3.3	0.0	*Valsa* sp.	5.0	3.7
Gibberella baccata	0.0	0.0	*Xylaria longipes*	0.3	2.7
Helotiales sp.	4.3	4.7	Xylariales sp. 1	5.0	5.0
Chaetomium globosum	0.0	5.0	Xylariales sp. 2	0.7	2.7
Leotiomycetes sp. 1	3.0	1.7			

Suppl. Table 2. Identification of isolates obtained from *Fraxinus excelsior* shoots based on molecular data (ITS rDNA) and morphology

Strain ID	Identification	Phenotype	Closest BLAST match	Accession Nr.	Identities (base pairs)
F1	*Valsa* sp.	*Cytospora* sp.	*Valsa* sp.	FJ228166	489/490
F2	*Sordaria fimicola*	*Sordaria fimicola*	*Sordaria fimicola*	AY681188	470/470
F3	*Phoma macrostoma*	*Phoma macrostoma* var. *macrostoma*	*Phoma macrostoma*	DQ474069	493/493
F4	*Aureobasidium pullulans*	*Aureobasidium pullulans*	*Aureobasidium pullulans*	JN886798	534/534
F5	*Phoma* sp.	*Phoma* sp.	*Phoma caloplacae*	JQ238635	526/547
F6	unidentified yeast	white yeast	DNA of poor quality		
F7	*Phoma macrostoma* var. *incolorata*	*Phoma macrostoma* var. *incolorata*	*Phoma macrostoma* var. *incolorata*	DQ474071	492/942
F8	*Gibberella baccata*	*Fusarium lateritium*	*Fusarium lateritium*	AF310980	518/518
F9	*Diaporthe* sp.	*Phomopsis* sp.	*Phomopsis quercina*	JX262803	534/534
F10	*Gibberella avenacea*	*Fusarium avenaceum*	*Fusarium avenaceum*	JX534353	512/512
F11	*Pyrenochaeta corni*	*Pyrenochaeta corni*	*Pyrenochaeta corni*	GQ387608	502/502
F12	Pleosporales sp. 1	yellowish to light olive coelomycete	Pleosporales sp.	JF449873	551/560

Suppl. Table 2. (Continued)

Strain ID	Identification	Phenotype	Closest BLAST match	Accession Nr.	Identities (base pairs)
F13	Pleosporales sp. 2	grey coelomycete, *Pyrenochaeta*-like	Pleosporales sp.	HQ207056	593/593
F15	*Desmazierella acicola*	*Desmazierella acicola*	*Desmazierella acicola*	LN589957	574/574
F16	*Penicillium citrinum*	*Penicillium* sp.	*Penicillium steckii*	HM469415	547/547
F17	*Lophiostoma corticola*	grey coelomycete	*Lophiostoma corticola*	AF383957	468/468
F18	*Alternaria alternata*	*Alternaria alternata*	*Alternaria alternata*	GQ328849	760/760
F20	*Prosthecium pyriforme*	*Stegonsporium pyriforme*	*Prosthecium pyriforme*	EU039975	522/526
F22	*Nemania serpens*	*Geniculosporium serpens*	*Hypoxylon serpens*	HM036598	538/538
F23	*Anthostomella pinea*	white mycelium	*Anthostomella pinea*	HQ599578	544/547
F24	*Cladosporium* sp.	*Cladosporium* sp.	*Cladosporium* sp.	KT270233	502/502
F25	*Botrytis cinerea*	*Botrytis cinerea*	*Botryotinia fuckeliana*	JQ693407	493/493
F27	*Bulgaria inquinans*	orange mycelium	*Bulgaria inquinans*	AY789345	472/473
F28	Dothideomycetes sp. 1	black-brown meristematic mycelium	*Endosporium aviarium*	EU304353	480/500
F29	*Aspergillus versicolor*	*Aspergillus versicolor*	*Aspergillus versicolor*	EF652478	663/663
F30	*Pleospora herbarum*	pale brown mycelium	*Pleospora herbarum*	GU584954	503/503
F31	*Aspergillus psudoglaucus*	*Aspergillus pseudoglaucus*	*Aspergillus pseudoglaucus*	FR839678	776/776
F32	*Rhodotorula mucilaginosa*	pink yeast	*Rhodotorula mucilaginosa*	AF444541	551/552
F34	*Chaetomium globosum*	*Chaetomium globosum*	*Chaetomium globosum*	JF773585	539/539
F38	Sordariomycetes sp. 1	ochre mycelium	Sordariomycetes sp.	JQ759839	443/458
F41	*Annulohypoxylon multiforme*	grey-brown mycelium	*Annulohypoxylon multiforme*	GU062284	500/500
F45	*Coniochaeta* sp.	yellow-orange mycelium	*Coniochaeta* sp.	JQ904605	543/543
F46	Dothideomycetes sp.2	pale grey coelomycete	Dothideomycetes sp.	JQ759636	504/516
F47	*Xylaria longipes*	white mycelium with brown margin	*Xylaria longipes*	AY909017	501/501
F52	*Annulohypoxylon cohaerens*	*Nodulisporium* sp.	*Annulohypoxylon cohaerens*	EF026140	566/566
F53	*Diaporthe* sp. 2	*Phomopsis* sp.	*Diaporthe cynaroidis*	EU552122	892/900
F57	*Lophiostoma* sp.	grey mycelium	*Lophiostoma* sp.	HE998729	414/414
F58	*Pezicula sporulosa*	*Cryptosporiopsis quercina*	*Pezicula sporulosa*	AF141166	521/523
F68	Sordariomycetes sp. 2	dark brown hyphomycete	Sordariomycetes sp.	KM519327	807/810
F69	Pleosporales sp. 3	olive mycelium with red pigment	*Phoma aliena*	KC311486	910/913
F71	Sordariomycetes sp. 3	dark brown hyphomycete	Sordariomycetes sp.	JQ760660	846/848
F75	*Prosthecium platanoidis*	brown-white coelomycete	*Prosthecium innesii*	JF681964	471/471
F81	*Coprinellus disseminatus*	pale yellow mycelium	*Coprinellus disseminatus*	JN159560	686/686
F89	Leotiomycetes sp. 1	white-orange coelomycete	Leotiomycetes sp.	JQ758675	690/720
F90	*Apiognomonia errabunda*	*Disculla umberinella*	*Apiognomonia errabunda*	AJ888477	550/551
F93	Dothideomycetes sp. 3	grey mycelium with olive-brown margine	Dothideomycetes sp.	HQ433040	794/823
F97	*Coniothyrium* sp.	*Coniothyrium* sp.	*Coniothyrium* sp.	EU852367	361/364
F98	Helotiales sp.	orange to ochre coelomycete	Helotiales sp.	HQ207037	585/618
F99	*Phaeomoniella* sp.	*Phaeomoniella* sp.	*Phaeomoniella* sp.	JN225891	341/343
F103	*Dendrothyrium* sp.	*Dendrothyrium* sp.	*Dendrothyrium variisporum*	JX496053	525/536
F106	Leotiomycetes sp. 2	white-orange hyphomycete	Leotiomycetes sp.	JQ758675	658/685
F108	Xylariales sp	white mycelium	*Anthostomella pinea*	HQ599578	449/474

Suppl. Table 2. (Continued)

Strain ID	Identification	Phenotype	Closest BLAST match	Accession Nr.	Identities (base pairs)
F109	Dothideomycetes sp. 4	white-brown coelomycete	Dothideomycetes sp.	JQ759636	538/585
F110	*Lopadostoma turgidum*	white mycelium	*Lopadostoma turgidum*	KC774617	627/630
F112	*Cryptodiaporthe hystrix*	*Diplodina acerina*	*Cryptodiaporthe hystrix*	EU255021	492/493
F114	Xylariales sp.2	white-brown mycelium	*Xylaria polymorpha*	AB512310	835/837
F115	*Bjerkandera adusta*	white mycelium	*Bjerkandera adusta*	FJ608590	736/736
F116	*Phialocephala* sp.	*Phialocephala* sp.	*Phialocephala* sp.	EU434850	341/347