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Species diversity of fungi on damaged branches and leaves of ashes (*Fraxinus* spp.) in different types of stands in Slovakia

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Abstract
The diversity of fungi on branches and leaves of ashes (*Fraxinus angustifolia*, *F. excelsior*, *F. ornus*) in Slovakia was studied. Symptomatic material collected in Slovakia during the period of 2013 to 2017 and herbarium specimens previously collected were examined. In total, 30 fungal taxa (15 Deuteromycetes, 14 Ascomycetes and one Basidiomycetes) were recorded. Twenty-three of them have never been recorded on ashes in the country. The most frequently occurring fungi were *Hymenoscyphus fraxineus* (anamorph *Chalara fraxinea*) that causes necrosis of shoots and branches, and *Phyllactinia fraxini*, a foliar pathogen that causes powdery mildew disease. Fungal diversity on ashes growing in different types of stands was compared. Species richness was the greatest in seed orchards (20 fungal taxa) compared to private gardens, which contained the lowest (two fungal taxa). Species diversity in forest stands comprised 18 fungal taxa and the urban greenery was represented by 10 fungal taxa. Nine fungal taxa were recorded in tree alley along the road. The widest fungal species spectrum was recorded on *F. excelsior*.

Key words: mycobiota; ash dieback; plant pathogens; saprophytes

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1. Introduction

Three native ash species, European ash (*Fraxinus excelsior* L.), narrow-leafed ash (*F. angustifolia* Vahl) and manna ash (*F. ornus* L.) occur on the territory of Slovakia. Ashes compose an important part of Slovak forests, and they are often planted as ornamental trees in the urban greenery. Although *Fraxinus* has relatively low representation in our forests (1.6%), ash wood remains industrially important (Anonymous 2015). This woody plant is sensitive to climatic fluctuations, especially to late frosts.

In the 1990s, an extensive outbreak of ash dieback occurred in north-eastern Europe. Due to the spread of the causal agent, *Hymenoscyphus fraxineus* (anamorph *Chalara fraxinea*) in recent years, ash trees of all ages are severely damaged across Europe. Some of the major symptoms of the disease are leaf necrosis, premature leaf abscission, and necrosis of the bark, cambium, and phloem, leading to the dying of shoots and branches. This disease causes a widespread mortality of ash trees. Thus, ash dieback threatens the existence of *Fraxinus* on the European continent (Vasaitis & Enderle 2017). *Fraxinus excelsior* has been registered in the Sweden's Red list of plants since 2010. *Hymenoscyphus fraxineus* attacks mainly *F. excelsior* and *F. angustifolia*. The following susceptible hosts have also been recorded: black (*F. nigra* Marshall), green (*F. pennsylvanica* Marshall), white (*F. americana* L.), Manchurian (*F. mandshurica* Rupr.), manna (*F. ornus*) and Chinese (*F. chinensis* subsp. *rhynchophylla* (Hance) E. Murray) ash trees (Drenkhan & Hanso 2010; Kirisits & Schwanda 2015; Gross & Han 2015). In Slovakia, the first evidence of the disease occurred more than 10 years ago (Kunca et al. 2006) and the identity of the fungal pathogen on *F. excelsior* and *F. angustifolia* was confirmed by molecular techniques (Adamčíková et al. 2015; Kádasi-Horáková et al. 2017). Due to the massive dieback of ashes at the present time, forest managers prefer culling or completely eliminating whole ash groups (Longauerová et al. 2017).

Although *H. fraxineus* is considered to be the main cause of dying ash trees, many other fungal genera (*Cytospora*, *Diplodia*, *Fusarium*, *Phomopsis*) colonizing ash shoots and branches are associated with their damage (Griffith & Boddy 1988; Przybyl 2002; Kowalski & Łukomska 2005; Kowalski & Czekaj 2010; Kowalski

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et al. 2016). In Slovakia, previous authors (Juhásová et al. 2003, 2004) recorded 10 fungal taxa on ashes during the assessment of the status of woody plant health in urban greenery. There has been no further data on the detailed composition of mycobiota of ashes in Slovakia recorded since the first appearance of the ash dieback in the country.

The aim of the present study was to determine species diversity of fungi colonizing and damaging branches and leaves of our native *Fraxinus* species growing in different types of stands in Slovakia.

2. Material and methods

From 2013 to 2017, symptomatic ash branches and leaves (*F. angustifolia*, *F. excelsior*, *F. ornus*) were collected at selected localities in the urban and extra-urban vegetation of Slovakia. Samples (one sample was material taken from one tree) were examined by means of a stereo microscope (Olympus SZ61, Tokyo, Japan) and standard light microscope (Olympus BX51, Tokyo, Japan). Fungi that have formed reproductive structures on the studied material were identified on the basis of their morphological characteristics using taxonomic manuals for fungi (Arx & Müller 1954; Dennis 1978; Sutton 1980; Sivanesan 1984; Ellis & Ellis 1985; Kiffer & Morelet 2000).

In addition to the direct identification of mycobiota on the examined material, an *in vitro* cultivation on artificial culture media was used to detect the presence of microscopic fungi. The selected material was surface-sterilized using 70% ethanol for 1 min, 2% sodium hypochlorite for 10 min, flushed with sterile distilled water, and then cultured on culture medium (potato-dextrose agar). The cultures were incubated in the dark at 22 ±2 °C in a climate chamber MLR-351H (Sanyo, Japan). Isolated fungi were identified based on their cultural and morphological characteristics using the above literature.

In order to find out the spectrum of fungi previously found on *Fraxinus* spp. in Slovakia in the past (before 2013), herbarium specimens deposited in the Plant Pathology Herbarium (herbarium code: NR) of the Institute of Forest Ecology SAS Zvolen, Branch for Woody Plant Biology in Nitra were examined and revised. Herbarium specimens were collected by Gabriela Juhásová after 1980.

Based on the sampling sites, the samples were categorized according to the type of stand (A – tree alley along the road, F – forest, G – private gardens, O – seed orchard and arboretum, P – public parks and inter-block greenery). The frequency (%) of occurrence of the fungal taxa was calculated.

3. Results

In our study, we focused on identifying microscopic fungi associated with damage of ash leaves and branches, as well as withering of ashes in forests, orchards and urban vegetation. A total of 287 samples were analysed. Samples were collected from branches and leaves of *Fraxinus* spp. growing at 82 sampling sites in different parts of Slovakia (Fig. 1). The sampling sites represented different types of ash stands with different levels of management (42 public parks and inter-block greenery, 18 forest stands, 14 tree alleys, 4 seed orchards including one arboretum, and 4 private gardens).

A total of 30 fungal taxa (15 Deuteromycetes, 14 Ascomycetes, and 1 Basidiomycetes) were identified on branches and leaves of *F. angustifolia, F. excelsior*, and *F. ornus*. The data overview and the frequency of the recorded fungi was given in Table 1. Twenty-three of the fungal taxa

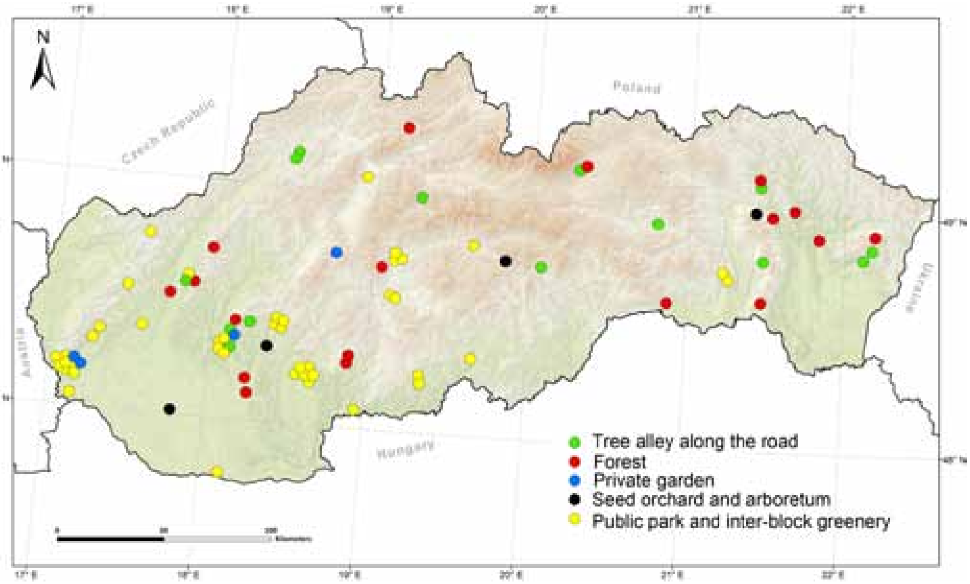

Fig. 1. Localization of sampling sites in Slovakia.

had never been recorded on *Fraxinus* in Slovakia. The novel taxa were marked with an asterisk (*) in Table 1. Fifteen species, namely *Botryosphaeria dothidea, Camarosporium orni, Coniothyrium fuckelii, Cryptosphaeria eunomia, Cucurbitaria obducens, Diaporthe eres, Didymosphaeria decolorans, Diplodia fraxini, Discula fraxinea, Dothiorella sarmentorum, Gloeosporidiella turgida, Hypoxylon fraxinophilum, Hysterographium fraxini, Immotthia atrograna*, and *Microdiplodia fraxini*, were not included in the checklist of fungi of Slovakia (Lizoň & Bacigálová 1998). The present paper documents the occurrence of these fungal species for the first time in Slovakia.

Under the *in vitro* conditions, the following 12 fungal taxa were isolated from ash shoots and branches: *Alternaria alternata*, *Botryosphaeria dothidea*, *Botrytis cinerea*, *Camarosporium orni*, *Cladosporium cladosporioides*, *Clonostachys rosea*, *Coniothyrium fuckelii*, *Diplodia fraxini*, *Dothiorella sarmentorum*, *Fusarium* sp., *Phomopsis* sp., and *Sordaria* sp.

Species richness (20 fungal taxa) was the greatest in seed orchards (monoculture plots). There were 18 fungal species found on ashes growing in forest stands (both single-tree species and mixed-tree species forest plots), 10 species in public parks and inter-block greenery, nine species in tree alleys along the road, and two species in private gardens (Fig. 2). The most common fungi occurring in all types of ash stands were *Hymenoscyphus fraxineus* causing necrosis of ash shoots and branches, and *Phyllactinia fraxini* causing powdery mildews. *Hymenoscyphus fraxineus* occurred very sporadically in urban ash plantations, presumably due to low quality of maintenance of a stand. The occurrence of the major pathogen, *H. fraxineus*, in the ash stands lead to the progressive withering and death of the trees. Rarely (only in one type of stand), 16 fungal taxa were recorded. There were 28 fungal taxa that colonized the bark and branches and 4 taxa were found on leaves, while two of them (*H. fraxineus* and *Phomopsis* sp.) occurred on the bark, branches, and the leaves. The list of fungi found on three species of *Fraxinus* in Slovakia is shown in Table 2. The widest fungal species spectrum was recorded on *F. excelsior*. The results showed that, in necrotic ash tissue, numerous fungi may occur, although only a few species are very frequent. Known plant pathogenic fungi present in a stand of dead ashes, such as: *Botryosphaeria dothidea, Cytospora sp., Diaporthe eres, Diplodia fraxini, Gibberella baccata*, and *Tubercularia vulgaris*, may represent a noticeable threat to young or stressed and weakened ash trees. These fungi

Table 1. The frequency of occurrence of fungal taxa on *Fraxinus* spp. in different type of stands in Slovakia.

Fungus	Mode of life	Substrate	Number of sampling sites[a]	Number of samples[b]	Frequency[c] [%]	Type of stand[d]
Ascomycetes						
**Botryosphaeria dothidea* (Moug.) Ces. & De Not.	parasite	branch	1	1	0.3	O
**Cryptosphaeria eunomia* (Fr.) Fuckel	parasite	branch	3	4	1.4	A, F, O
**Cucurbitaria obducens* (Schumach.) Petr.	saprophyte	branch	3	3	1.0	A, F
**Diaporthe eres* Nitschke	parasite	branch	2	2	0.7	F, P
**Didymosphaeria decolorans* Rehm	saprophyte	branch	1	3	1.0	F
Gibberella baccata (Wallr.) Sacc.	parasite	branch	1	1	0.3	P
Hymenoscyphus fraxineus (T. Kowalski) Baral, Queloz & Hosoya; anamorph *Chalara fraxinea* T. Kowalski	saprophyte, parasite	leaf, branch	25	120	41.8	A, F, G, O, P
**Hypoxylon fraxinophilum* Pouzar	saprophyte	branch	1	8	2.8	F
**Hysterographium fraxini* (Pers.) De Not.	saprophyte	branch	3	9	3.1	A, F, O
**Immotthia atrograna* (Cooke & Ellis) M.E. Barr	mycoparasite	branch	1	1	0.3	F
Neonectria ditissima (Tul. & C. Tul.) Samuels & Rossman (=*Nectria galligena* Bres.)	saprophyte	branch	3	6	2.1	F, O, P
Phyllactinia fraxini (DC.) Fuss (=*P. guttata* (Wallr.) Lév.)	parasite	leaf	41	73	25.4	A, F, G, O, P
**Sordaria* sp.	saprophyte	branch	1	2	0.7	O
**Xylaria longipes* Nitschke	saprophyte	branch	1	1	0.3	F
Basidiomycetes						
**Trametes versicolor* (L.) Lloyd	saprophyte	branch	1	1	0.3	P
Deuteromycetes						
**Alternaria alternata* (Fr.) Keissl.	endophyte	branch	1	2	0.7	O
**Botrytis cinerea* Pers.	endophyte	branch	1	1	0.3	O
**Camarosporium orni* Henn.	saprophyte	branch	3	5	1.7	A, F, O
**Cladosporium cladosporioides* (Fresen.) G.A. de Vries	endophyte	branch	1	1	0.3	O
**Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams	mycoparasite	branch	1	1	0.3	O
**Coniothyrium fuckelii* Sacc.	saprophyte	branch	1	1	0.3	O
**Cytospora* sp.	parasite	branch	5	15	5.2	A, F, O, P
**Diplodia fraxini* (Fr.) Fr.	parasite	branch	5	9	3.1	A, F, O, P
**Discula fraxinea* (Peck) Redlin & Stack	parasite	leaf	1	1	0.3	F
**Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, A. Alves & J. Luque	parasite	branch	1	2	0.7	O
Fusarium sp.	parasite	branch	1	1	0.3	O
**Gloeosporidiella turgida* (Berk. & Broome) B. Sutton	parasite	branch	3	3	1.0	F, O, P
**Microdiplodia fraxini* Died.	saprophyte	branch	1	1	0.3	F
Phomopsis sp. (=*Phyllosticta fraxinicola* Sacc.)	parasite	leaf, branch	6	7	2.4	F, O, P
Tubercularia vulgaris Tode	parasite	branch	2	2	0.7	A, O

[a] Number of sampling sites on which the occurrence of identified fungi was recorded.
[b] Number of samples in which the identified fungi were present (out of 287 examined samples).
[c] Frequency of the occurrence of fungi in total collection of 287 examined samples.
[d] A – tree alley along the road, F – forest, G – private gardens, O – seed orchard and arboretum, P – public parks and inter-block greenery.
*Fungal taxa not recorded on *Fraxinus* to date in the country.
The name on the original herbarium label is indicated in bracket.

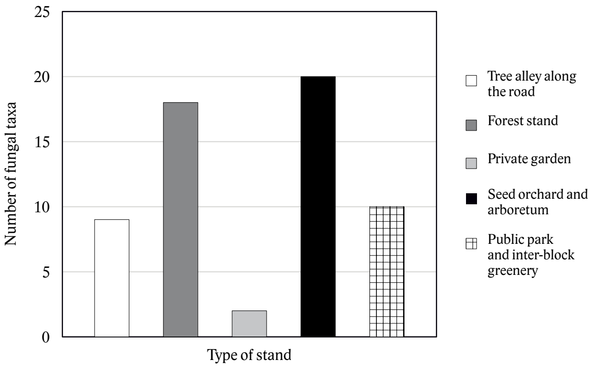

Fig. 2. Number of fungal taxa by type of ash stand in Slovakia.

possess a broad host spectrum that also places other tree species at risk of disease.

The fungi that we recorded on ash branches and leaves represent the taxa with different heterotrophy, such as saprophytes feeding on the dead or decaying substrates (e.g. *Cucurbitaria*, *Didymosphaeria*, *Hymenoscyphus*, *Hypoxylon*, *Hysterographium*, *Neonectria*, *Sordaria*, *Trametes*, *Xylaria*), parasites that prefer to colonize living plant tissues (e.g. *Chalara*, *Diplodia*, *Gloeosporidiella*, *Phomopsis*, *Phyllactinia*, *Tubercularia*), and endophytes (*Alternaria*, *Botrytis*, *Cladosporium*). Also mycoparasitic fungi, *Immotthia atrograna* parasitic on *Hypoxylon fraxinophilum* and *Clonostachys rosea* parasitic on *Sordaria* sp., were recorded on dead branches of *F. excelsior*.

Fungal specimens obtained during this study were preserved as a collection of herbarium materials (NR), as a data source for studying the geographical distribution of fungi and as the source of DNA of the fungi for molecular taxonomic analyses.

Table 2. The spectrum of fungi found on *Fraxinus* spp. in Slovakia.

Host	Fungus	Location of sampling sites
F. angustifolia	*Alternaria alternata*	Trstice[c]
	Botryosphaeria dothidea	Trstice[c]
	Botrytis cinerea	Trstice[c]
	Camarosporium orni	Trstice[c]
	Cladosporium cladosporioides	Trstice[c]
	Coniothyrium fuckelii	Trstice[c]
	Diplodia fraxini	Trstice[c]
	Dothiorella sarmentorum	Trstice[c]
	Fusarium sp.	Trstice[c]
	Hymenoscyphus fraxineus	Trstice[b], Vieska nad Žitavou[b]
	Hysterographium fraxini	Trstice[b]
	Phomopsis sp.	Trstice[c]
	Phyllactinia fraxini	Bratislava[a,b], Nitra[a,b]
	Sordaria sp.	Trstice[c]
F. excelsior	*Alternaria alternata*	Trstice[c]
	Camarosporium orni	Považská Teplá[b], Stará Lesná[b]
	Clonostachys rosea	Trstice[c]
	Cryptosphaeria eunomia	Kuková[b], Podhorany (Nitra District)[b], Vieska nad Žitavou[b]
	Cucurbitaria obducens	Kuková[b], Podhorany (Nitra District)[b], Stará Lesná[b]
	Cytospora sp.	Kuková[b], Nitra[b], Piešťany[b], Podhorany (Nitra District)[b], Stará Lesná[b]
	Diaporthe eres	Nitra[b], Stará Lesná[b]
	Didymosphaeria decolorans	Kuková[b]
	Diplodia fraxini	Kostoľany pod Tríbečom[b], Kuková[b], Nitra[b], Piešťany[b]
	Discula fraxinea	Kuková[b]
	Dothiorella sarmentorum	Trstice[c]
	Gibberella baccata	Nitra[a]
	Gloeosporidiella turgida	Kuková[b], Trstice[b]
	Hymenoscyphus fraxineus	Badín[b], Brekov[b], Černík[b], Hermanovce nad Topľou[b], Hôrka nad Váhom[b], Kuková[b], Kvakovce[b], Ladzany[b], Lipníky[b], Podsuchá[b], Poruba pod Vihorlatom[b], Považská Teplá[b], Remetské Hámre[b], Štitáre[b], Topoľčianky[b], Trstice[b], Turňa nad Bodvou[b], Úľany nad Žitavou[b], Vieska nad Žitavou[b], Zázrivá[b], Zbojská[b]
	Hypoxylon fraxinophilum	Kuková[b]
	Hysterographium fraxini	Kuková[b], Stará Lesná[b], Trstice[b]
	Immotthia atrograna	Kuková[b]
	Microdiplodia fraxini	Stará Lesná[b]
	Neonectria ditissima	Kostoľany pod Tríbečom[b], Kuková[b]
	Phyllactinia fraxini	Banská Bystrica[a], Bratislava[a,b], Brezno[b], Handlová[a], Herľany[a], Horné Lefantovce[a], Komárno[a], Košice[a], Levice[a], Modra[a], Muráň[b], Myjava[a], Nitra[a,b], Pezinok[a], Slanec[a], Šahy[a], Topoľčianky[b], Trnava[a], Trstice[a], Trstín[b], Veľké Kostoľany[a], Vieska nad Žitavou[a,b], Zvolen[a]
	Phomopsis sp. *(=Phyllosticta fraxinicola)*	Banská Bystrica[a], Košice[a], Kuková[b], Nitra[b], Vieska nad Žitavou[a]
	Sordaria sp.	Trstice[c]
	Trametes versicolor	Pezinok[a]
	Tubercularia vulgaris	Spišské Vlachy[b], Trstice[b]
	Xylaria longipes	Považský Inovec[b]
F. ornus	*Gloeosporidiella turgida*	Bratislava[a]
	Hymenoscyphus fraxineus	Vieska nad Žitavou[b]
	Neonectria ditissima (=Nectria galligena)	Bratislava[a]
	Phyllactinia fraxini	Bratislava[a,b], Martin[a], Nitra[a,b], Veľký Krtíš[a]

[a] Herbarium specimens deposited in the Plant Pathology Herbarium (NR) collected before 2013.
[b] Samples collected in 2013–2017.
[c] Fungal isolates on culture media (*in vitro*).
The name on the original herbarium label is indicated in bracket.

4. Discussion

The results show that the mycobiota associated with ash trees in Slovakia are very rich in species diversity. In previous papers (Juhásová et al. 2003, 2004), 10 fungal taxa on ashes growing in urban greenery have been recorded. Twenty-three other fungal taxa on ashes growing in five different types of stands were recorded in the present paper. Representative material of previously published findings of some fungi on ashes from Slovakia, *Cercospora fraxini* Ellis & Kellerm., *Gibberella baccata* (anamorphic stage of *Fusarium lateritium* Nees) and *Mycosphaerella fraxini* (Niessl) Lindau (Juhásová et al. 2003, 2004) were not preserved. We did not record any wood-decaying fungi on the ashes examined from 2013 to 2017. The only herbarium specimen, *Trametes versicolor*, on *F. excelsior* is stored in NR herbarium. *Ganoderma adspersum* (Schulzer) Donk, *G. applanatum* (Pers.) Pat. (=*G. lipsiense* (Batsch) G.F. Atk.), *G. carnosum* Pat., and *G. pfeifferi* Bres. have been recorded on *Fraxinus* spp. in Slovakia (Juhásová et al. 2003; Gašparcová et al. 2017). The basidiomycete *Inonotus hispidus* (Bull.) P. Karst. causes an intense white rot and ash wood degradation in Slovak forests (Zúbrik et al. 2017). Longauerová et al. (2013) recorded an occurrence of *Armillaria cepistipes* Velen. and *A. gallica* Marxm. & Romagn. on trees weakened by *H. fraxineus*. Wilt disease caused by *Verticillium dahliae* Kleb. and leaf blotch caused by *Kabatiella apocrypta* (Ellis & Everh.) Arx, responsible for the mortality of ash trees in nurseries in Germany (Schröder & Dujesiefken 2001), were not recorded in Slovakia. In our study, we noticed a rare occurrence of the fungus *Discula fraxinea* that causes anthracnose disease of ashes, premature leaf abscission, and the defoliation and disfigurement of infected branches in North America (Jacobs & Danielson 2002).

In an extensive study of the mycobiota in declining ashes in Polish forests, Kowalski et al. (2016) identified more than 70 fungal taxa on stems and twigs in initial and advanced stages of dieback. They recorded *H. fraxineus* in almost 60% of the samples analysed, as well as the following frequently occurring fungi: *Alternaria alternata*, *Diaporthe eres*, *Diplodia mutila* (Fr.) Mont., *Fusarium avenaceum* (Fr.) Sacc., *F. lateritium*, and *Phomopsis* spp. Griffith & Boddy (1988) recorded the species *Phomopsis platanoidis* (Cooke) Died., *Libertella fraxinea* Oganova, *Peniophora lycii* (Pers.) Höhn. & Litsch., *F. lateritium* and *Acremonium* sp. on dead branches in ash crowns. Bakys et al. (2009) confirmed the pathogenicity in four of 24 isolated fungal species and reported symptomatic necrosis of the bark and cambium were caused by *A. alternata*, *Epicoccum nigrum* Link, *Chalara fraxinea* and *Phomopsis* sp. Przybyl (2002) observed the necrosis of the tissue on young *Fraxinus* seedlings inoculated with *Diplodia mutila* and *F. solani* (Mart.) Sacc. *Diplodia fraxini*, which we isolated from *F. angustifolia* and *F. excelsior* collected from five localities in Slovakia, is a species belonging to the *Diplodia* species complex associated with cankers and branch dieback on *Fraxinus* spp. in Europe. Alves et al. (2014) recorded *D. mutila*, *D. pseudoseriata* C.A. Pérez, Blanchette, Slippers & M.J. Wingf., *D. seriata* De Not., *D. subglobosa* A.J.L. Phillips, Deidda & Linald. and *D. fraxini* on all three native *Fraxinus* species. In the taxonomic revision of the genus *Phyllosticta*, van der Aa & Vanev (2002) re-examined the material previously published as *Phyllosticta fraxini* Ellis & G. Martin and *Phyllosticta fraxinicola* on *Fraxinus* spp. and designated the taxa as *Phomopsis* sp., the anamorphic stage of *Diaporthe eres*. The pathogenicity of *Phomopsis controversa* (Desm.) Traverso and *P. scobina* Höhn. isolated from necrotic ash shoots have not been confirmed (Przybyl 2002).

Powdery mildew disease caused by *Phyllactinia fraxini* occurs commonly on all three native species of *Fraxinus* in Slovakia (Table 2). Paulech (1995) recorded *P. fraxini* also on *F. americana* (Hurbanovo, Sesíleš, park). An Asian powdery mildew fungus, *Erysiphe salmonii* (Syd. & P. Syd.) U. Braun & S. Takam., recently introduced to Europe and found on *F. excelsior* and *F. pennsylvanica* in Ukraine (Heluta et al. 2017), was not recorded in our country until now.

Diverse forests can contribute to reduced susceptibility of trees to disease and fungal infection, and a subsequent increase in plant survival and growth (Keesing et al. 2006; Hantsch et al. 2014). According to Keesing et al. (2006), non-host trees can reduce fungal disease risks. When the density of these heterospecific trees increases, the proportion of host trees then becomes diluted in mixed stands. Our results showed a richer diversity of fungal taxa in seed orchards (monoculture plots) and both single-tree species and mixed-tree species forest plots. The invasive pathogenic fungus, *H. fraxineus*, caused devastating damage to the ashes growing in forests, seed orchards and tree alleys. We assume that very sporadic findings of *H. fraxineus* in urban ash plantations are a consequence of the quality of maintenance of urban greenery. The presence of the pathogen confirmed in seeds of *F. excelsior* (Cleary et al. 2013) is of great concern to phytosanitary protection authorities in countries outside the current zone of infestation.

Ash with extensive dieback symptoms rarely recovers under field conditions. There is very low proportion of trees tolerating infection by *H. fraxineus* in current common ash populations. Clonal seed orchards composed of dieback-tolerant clones appear to be the most efficient tool for management of ash dieback. The results of experiments on the selection and testing of candidate hyposensitive clones for new ash seed orchards are only preliminary (Longauerová et al. 2017). There have been early steps in propagating and screening a wide range of *Fraxinus* species and selection of tolerant *F. excelsior* genotypes for a new breeding program (Clark & Webber 2017). There is currently no information on an effective control method for *H. fraxineus*. The maintenance of high tree vigor using cultural practices such as destroying fallen diseased leaves, pruning out dead branches and covering wounds with fungicide-augmented dressings is recommended.

5. Conclusion

The results of species diversity of fungi colonizing three native *Fraxinus* species in Slovakia are presented. A rich diversity of fungi on ashes represents a total of 30 fungal taxa. Although numerous fungi occur in necrotic ash tissue, only a few taxa are very frequent. Some of plant pathogenic fungi that are present in the stand of dead ashes may represent a noticeable threat to young or stressed and weakened ash trees. Other tree species may also be at risk of disease since these pathogenic fungi possess broad host spectrums. We assume other species of pathogenic fungi could also be present in dying ashes and thus contribute to dieback. Further mycological surveys are needed to identify fungal species that benefit from the initial infection by *H. fraxineus* or contribute to progress of ash dieback disease.

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