Research paper

Decline of *Fraxinus excelsior* L. in parks of Saint Petersburg: Who is to blame – *Hymenoscyphus fraxineus* or *Diplodia* spp.?

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Abstract. The weakening and decline of European ash *Fraxinus excelsior* L. and other ash species have been recorded at different locations in the suburbs of Saint Petersburg, Russia. During the summer of 2019 and spring of 2020, samples from leaves, petioles, and shoots were collected from the weakened and declining ash trees in three parks in Pushkin and Gatchina and maintained in humid chambers to induce the fructification of fungi. In total, 30 taxa of micromycetes belonging to 23 genera were identified using methods of light microscopy. *Hymenoscyphus fraxineus*, a putative agent of ash dieback, was not recorded in the samples collected in the crowns of trees, but only on the petioles of the fallen leaves in spring. Out of all the micromycetes recorded, only coelomycetes from the genus *Diplodia* Fr. (in particular, *D. mutila*) can damage the branches of ash trees and, thus, be considered pathogenic. It is likely that *H. fraxineus* opens “the entry of infection” and *Diplodia* spp. cause the major weakening and decline of branches. The data obtained can significantly change our understanding of the causes of ash dieback and possible methods of ash stand preservation. The reason for the low pathogenicity and activity of *H. fraxineus*, as well as the possible role of ascomycetes *Diplodia* spp. in the dieback of ash stands requires further research.

Key words: ash dieback, European ash, Ascomycota, tree health, invasive tree fungal pathogen, urban greening.

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Introduction

Urban and suburban green spaces fulfill a very important function of creating a comfortable living environment. In large European cities and urban agglomerations,

tree stands are usually formed by species of woody plants that are not characteristic of the surrounding local forest ecosystems. This is conditioned by the specific features of the urban environment; predominantly, the aesthetic requirements and the im-

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part of the negative anthropogenic factors, such as air and soil pollution (Nowak *et al.*, 2006; Alekseyev *et al.*, 2019). For example, city parks and urban plantings of Saint Petersburg, Russia, are mostly planted by the small-leaved lime *Tilia cordata* Mill., Norway maple *Acer platanoides* L., silver birch *Betula pendula* Roth, Berlin poplar *Populus × berolinensis* Dippel, green ash *Fraxinus pennsylvanica* Marsh, English oak *Quercus robur* L., and European white elm *Ulmus laevis* Pall., whereas the forest ecosystems of Saint Petersburg suburbs and the surrounding Leningrad Province are dominated by Scots pine *Pinus sylvestris* L., Norway spruce *Picea abies* (L.) H. Karst., a few species of birch *Betula* spp., European aspen *Populus tremula* L., and grey alder *Alnus incana* (L.) Moench (Moschenikova, 2011). The specific composition of woody vegetation and a strong influence of the factors compromising the resistance of woody plantings make these plantings highly susceptible to invasive pathogens and pests in the urban environment (Selikhovkin *et al.*, 2018, 2020).

Saint Petersburg is a big cultural center that attracts millions of tourists every year and also serves as a transportation hub. As a result, the process of invasion of pathogens and arthropod pests of woody plants is very intensive here (Shabunin *et al.*, 2012; Musolin *et al.*, 2017; Selikhovkin *et al.*, 2018, 2020). The ascomycete *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya (anamorph of *Chalara fraxinea*) causing ash dieback is one of the phytopathogenic invaders that recently reached the city. During the last 20 years, this fungus has rapidly spread over the most of Europe causing fast and almost complete death of ash stands everywhere it was recorded (Enderle *et al.*, 2017; Vasaitis & Enderle, 2017). This species was recorded in Saint Petersburg as well, in particular, at Dudergof Heights in the drying-off stands of European ash *Fraxinus excelsior* L. (Shabunin *et al.*, 2012).

During recent routine surveys of parks

and urban plantings in Saint Petersburg and its suburbs, deterioration of the health conditions of ash trees was noted: individual shoots were dying off and individual trees or groups of trees died in Aleksandrovsky and Babolovsky Parks of Pushkin district of Saint Petersburg, as well as in Gatchina Palace Park in Leningrad Province (Figure 1). The dying trees often had symptoms characteristic of ash dieback induced by *H. fraxineus* (Vasaitis & Enderle, 2017). Thus, it was suggested that invasive *H. fraxineus* might be a pathogenic agent causing death of ash trees in these parks.

The aim of the study was to evaluate a possible role of *H. fraxineus* as well as other pathogenic fungi in the death of ash trees in the parks of Saint Petersburg and its suburbs.

Material and Methods

In the second half of July and in August of 2018 and 2019, and in May 2020 surveys were conducted in Aleksandrovsky Park (59°43'30" N, 30°22'20" E) and Babolovsky Parks (59°43'03" N, 30°22'11" E) of Pushkin district of Saint Petersburg as well as in Gatchina Palace Park (59°33'51" N, 30°06'49" E) in Leningrad Province (Figure 1). We evaluated the health conditions of European ash *F. excelsior* trees (aged 30 years and older; breast height diameter of 20 cm and more). In Aleksandrovsky Park, 37 ash trees that looked the most weakened were checked and evaluated, in Babolovsky Park – 35 trees, and in Palace Park in Gatchina – 88 ash trees (Figure 2). In Aleksandrovsky and Babolovsky Parks, ash dieback tended to have a cluster character when diseased trees formed a group of near-growing ash trees (Figure 2).

To evaluate the health condition of ash trees, we used standardized categories adopted in the Russian Federation for forest pathology surveys using six categories: (I) healthy trees, (II) weakened trees, (III) heavily weakened trees, (IV) drying-out

Figure 1. Location of the parks surveyed in this study.

Figure 2. Group weakening and decline of European ash *Fraxinus excelsior* trees in Babolovsky Park (photo by Bui Dinh Duc, with permission).

trees, (V) trees that had died during the current year, and (VI) trees that had died during the previous year (categories V and VI were combined in our surveys; Mozolevskaya *et al.*, 1984).

In 2019, a focused study was conducted in the same locations with a purpose to detect the presence and identify phytopathogenic fungi causing deterioration of the ash trees' health status. Three times during the summer season (on June 26, July 25, and August 27), we collected dried and damaged shoots with leaves from the lower part of the crown of adult ash trees using a 5.5 m long rod secateurs. The last year's fallen leaf petioles were collected on July 25, 2019. The next year, the collection of the petioles was repeated on May 27, 2020. In most cases, samples of leaves and shoots

from crowns were collected from the same trees on all three dates. In total, 30 samples were collected from 20 trees (namely, 11 samples from 9 trees in Aleksandrovsky Park, 5 samples from 3 trees in Babolovsky Park, and 14 samples from 8 trees in Palace Park). Samples were collected mostly from heavily weakened trees (health category III: 23 samples from 17 trees; Figure 3), weakened trees (health category II: 5 samples from 2 trees) followed by drying-out trees (health category IV: 2 samples from 1 tree).

The sampled leaves, petioles, and shoots were studied in a laboratory using stereomicroscope MBS-9. Then, the samples were incubated in a humid chamber under room conditions with regular monitoring of the emerging fruit bodies of micromycetes. After 3–4 weeks of incubation, in most cases, all samples were covered with sterile mycelium and the observations ceased.

The identification of fungal species was carried out during the monitoring of the appearance of micromycetes fruiting in the humid chamber using morphological characteristics under the upright microscope Motic BA400. The appearance of the putative pathogen *H. fraxineus* was monitored by observing the development of fungi in the humid chamber, as it was previously revealed that in the humid chamber fruiting of the imperfect stage of *H. fraxineus* develops quite quickly (Shabunin *et al.*, 2012; Musolin *et al.*, 2017).

Results

In all three parks (Aleksandrovsky, Babolovsky, and Palace Park), the group weakening and death of European ash trees having the symptoms characteristic of ash dieback caused by *H. fraxineus* on the crowns' periphery were observed. All the surveyed ash trees had symptoms of weakening, including the drying-out of peripheral shoots, individual branches,

Figure 3. Typical appearance of European ash *Fraxinus excelsior* of the health category III (heavily weakened trees) from which samples were collected for the identification of pathogenic micromycetes (Aleksandrovsky Park, Pushkin; photo by Andrey V. Selikhovkin).

Table 1. The health state of *Fraxinus excelsior* trees in the parks of Pushkin and Gatchina according to the surveys of 2018–2019.

Health state categories of trees	Score	Number of trees in each category for each park (%)		
		Aleksandrovsy Park	Babolovsky Park	Palace Park
I (healthy trees)	1	0 (0.0)	0 (0.0)	0 (0.0)
II (weakened trees)	2	5 (13.5)	3 (8.6)	16 (18.2)
III (heavily weakened trees)	3	20 (54.1)	12 (34.3)	54 (61.4)
IV (drying-out trees)	4	10 (27.0)	15 (42.8)	15 (17.0)
V (trees that had died during the current year) and VI (trees that had died during the previous year) combined	5 and 6	2 (5.4)	5 (14.3)	3 (3.4)
Total		37 (100)	35 (100)	88 (100)
Weighted score of the tree health category		3.25	3.70	3.07

and, in some cases, significant sections of the crowns. Dead trees were recorded in all parks (Table 1). On average, the health state score of ash stands was between heavily weakened trees (category III) and drying-out trees (IV), with the situation in Babolovsky Park being the worst (Table 1).

Monitoring of the incubation of the collected samples in the humid chamber allowed to identify 30 taxa of micromycetes belonging to 24 genera (Table 2). We recorded 19 taxa of micromycetes belonging to 14 genera from the leaf tissues, 13 species (10 genera) from petioles, and 9 taxa (8 genera) from shoots (Table 2). All the registered fungal species are typical for the ash mycobiota. The coelomycetes of the genus *Diplodia* were found throughout the entire observation period and were the most prevalent species.

The ascomycete *H. fraxineus*, a putative agent of ash dieback, was not found in the collected samples from the crowns of *F. excelsior*. The fungus was not also found on petioles of the fallen leaves collected on July 25, 2019. Only in the next spring, typical dark brown pseudosclerotial tissues were observed. During the subsequent incubation in a humid chamber, fruiting bodies of the fungus appeared on the tissue samples that had overwintered on soil

and had been collected on May 27, 2020.

In contrast, fungi of the genus *Diplodia* were found in all three parks and during all three months in 2019, although not at each sampling collection session (Table 2). Based on morphological characteristics, the samples collected in the beginning and in the middle of summer were identified as *Diplodia mutila* (Fr.) Mont. (Figure 4). In the samples collected in August 2019, conidia had a slightly larger size and somewhat different pigmentation. These specimens were identified only up to the genus level (*Diplodia* sp.). All other fungi detected on the collected samples do not induce observed crown damage (Table 2).

Discussion

The conducted survey demonstrated that symptoms of the weakening and dieback of ash that are usually associated with *H. fraxineus* were found in many ash trees and in all three parks. In fact, there were absolutely no healthy ash trees among surveyed trees, and the average tree health score ranged from 3.07 (heavily weakened trees) to 3.7 (almost drying-out trees). However, in our samples from tree crowns, *H. fraxineus* was not found in any

Table 2. Micromycetes from the leaves, petioles, and shoots of *Fraxinus excelsior* cultivated in a humidified incubator.

Date of sample collection	Location of sampling and identified micromycetes		
	Aleksandrovsky Park	Babolovsky Park	Palace Park
June 26, 2019	* <i>Codinaea</i> sp. *** <i>Diplodia mutila</i> (Fr.) Mont.	* <i>Phomopsis</i> sp. * <i>Stachybotrys</i> sp.	* <i>Coniothyrium</i> sp. *** <i>Diplodia mutila</i> (Fr.) Mont.
July 25, 2019	* <i>Cladosporium herbarum</i> (on galls of <i>Dasineura fraxinea</i>) *** <i>Diplodia mutila</i> (Fr.) Mont.	* <i>Cladosporium herbarum</i> (on galls of <i>Dasineura fraxinea</i>) * <i>Phyllactinia fraxini</i> (DC.) Fuss *** <i>Hypoxyton fraxinophilum</i> Pouzar.	* <i>Cladosporium herbarum</i> (on galls of <i>Dasineura fraxinea</i>) * <i>Coniothyrium</i> sp. * <i>Trichothecium roseum</i> (Pers.) Link. ** <i>Marasmius</i> sp. ** <i>Phomopsis scobina</i> (Cooke) Höhn. *** <i>Coniothyrium</i> sp. *** <i>Diplodia mutila</i> (Fr.) Mont. *** <i>Trichothecium roseum</i> (Pers.) Link.
August 27, 2019	* <i>Alternaria</i> sp. * <i>Botrytis</i> sp. (<i>B. cinerea</i> ?) * <i>Cladosporium</i> spp. * <i>Gonatobotrys flava</i> Bonord. * <i>Hansfordia</i> sp. * <i>Harsia</i> sp. * <i>Periconia cookei</i> E.W. Mason et M.B. Ellis * <i>Periconia</i> sp. * <i>Phomopsis scobina</i> (Cooke) Höhn. * <i>Stachybotrys</i> sp. * <i>Trichothecium roseum</i> (Pers.) Link. *** <i>Diplodia</i> sp. *** <i>Gliomastix</i> sp.	* <i>Phyllactinia guttata</i> *** <i>Diplodia</i> sp. *** <i>Cytospora pruinosa</i> (Fr.) Sacc. *** <i>Trichoderma</i> sp.	* <i>Alternaria</i> sp. * <i>Cladosporium</i> spp. * <i>Gliomastix</i> sp. * <i>Periconia</i> sp. * <i>Phomopsis scobina</i> (Cooke) Höhn. * <i>Phyllactinia</i> sp. (possibly, <i>P. guttata</i>) *** <i>Diplodia</i> sp. *** <i>Stachybotrys</i> sp. *** <i>Trichoderma</i> sp.
May 27, 2020		** <i>Alternaria</i> spp. ** <i>Cladosporium</i> sp. ** <i>Fusicladium fraxini</i> Aderh. ** <i>Hymenoscyphus fraxineus</i> (T. Kowalski) Baral, Queloz & Hosoya	** <i>Alternaria</i> sp. ** <i>Cladosporium macrocarpum</i> Preuss ** <i>Cladosporium</i> sp. ** <i>Coniothyrium</i> sp. ** <i>Dictyochoeta</i> sp. ** <i>Fusicladium fraxini</i> Aderh. ** <i>Gyothrix ramosa</i> Zucconi & Onofri ** <i>Hymenoscyphus fraxineus</i> (T. Kowalski) Baral, Queloz & Hosoya ** <i>Periconia cookei</i> E.W. Mason & M.B. Ellis ** <i>Periconia</i> sp.

Notes: * – sampled on leaves; ** – sampled on petioles; *** – sampled on shoots.

Figure 4. Conidia (left), conidia and conidiophores (right) of *Diplodia mutila* (Fr.) Mont. from shoots of *Fraxinus excelsior* collected on July 25, 2019 in Aleksandrovsky Park, Pushkin (left) and Palace Park, Gatchina (right) (photo by Dmitrii A. Shabunin).

of the surveyed park and on any date. The fungus was found only on the petioles of fallen leaves in spring. This finding suggests that the role of the highly pathogenic *H. fraxineus* in the weakening and dieback of ash crowns in the surveyed parks is questionable.

Out of the 30 taxa of micromycetes belonging to the 23 genera recorded in this study (Table 2), only coelomycetes from the genus *Diplodia* Fr. can cause shoot blight of such a kind (Merezhko, 1980). A few species of *Diplodia* are known to be associated with ash species (Alves *et al.*, 2014). Among them, *D. mutila* is the most reported species (Alves *et al.*, 2014; Kowalski *et al.*, 2016). We identified this species among the samples collected in June and July 2019 in Aleksandrovsky and Babolovsky Park (Table 2). In the samples collected in August 2019 in all three parks, *Diplodia* sp. was also present, but we failed to identify particular species. On average, *Diplodia* sp. was recorded on 37.5–50.0% ash trees in the survey of 2019.

It is interesting to note that phytopathogenic *Diplodia* spp. were not recorded in the ash stands severely damaged by *H. fraxineus* in the Tellerman Experimental Forestry located in Voronezh Province, Russia (Kolganikhina, 2018).

The pathogenicity of *H. fraxineus* is known to depend on various factors, in-

cluding landscape characteristics (Grosdidier *et al.*, 2020). However, the data obtained in Pushkin and Gatchina can significantly change our understanding of the causes of ash dieback and possible methods for the preservation of ash stands. The local foci of ash dieback caused by *H. fraxineus* were recorded in ash plantations at Duderhof Heights (Shabunin *et al.*, 2012) which is only 14–18 km away from Pushkin and Gatchina. It is also known that the presence of *H. fraxineus* was confirmed in 2011 in Saint Petersburg, where ash leaf petioles with apothecia of the fungus were found in the Botanical Garden (Dendrarium) of Saint Petersburg State Forestry Technical University and Botanical Garden of the Botanical Institute of the Russian Academy of Sciences (Musolin *et al.*, 2014, 2017). Despite the pathogen's presence, the decline of ash trees was not obvious at that time and is not observed now. These foci of *H. fraxineus* are only 30–50 km northward from Pushkin and Gatchina. Given the fairly close distance between all these points, the low activity and pathogenicity of *H. fraxineus* in Pushkin and Gatchina are interesting facts that require further research.

Under the circumstances when *H. fraxineus* was not recorded in the samples collected from the ash trees with the symptoms

of dieback, the presence of *Diplodia* spp. can indicate that these pathogenic ascomycetes cause the weakening and death of ash trees in the surveyed parks. However, data are scarce on the pathogenicity of *Diplodia* spp. developing on deciduous woody plants in general and on ash in Russia in particular. There is evidence of the pathogenicity of these fungi to conifers, but the issue is poorly studied in respect of ash species (Merezhko, 1980; Zhukov *et al.*, 2013). Elsewhere, it has been reported that young ash trees died because of spring frosts followed by infection by *Diplodia mutila*, *Gloeosporidiella turgida* (Berk. & Broome) B. Sutton, and *Phomopsis scobina* (Cooke) Höhn. in Austria in 2005 (Cech, 2006). Also, *D. mutila* was isolated from the affected shoots of ash in Poland (Przybył, 2002; Kowalski & Łukomska, 2005) as well as in other European countries (Alves *et al.*, 2014).

Conclusions

The pathogenic ascomycete *H. fraxineus* was not found in the samples collected from the crowns of the weakened and heavily damaged *F. excelsior* trees in the parks of Pushkin and Gatchina. It was recorded only on the overwintered petioles of fallen leaves. At the same time, the pathogenic ascomycetes *Diplodia* spp. and, in particular, *D. mutila* colonized almost all shoots sampled. Under such circumstances, we assume that *H. fraxineus* opens “the entry of infection”, and *Diplodia* spp. cause the main weakening and decline of branches.

The data obtained suggest that there are some specific factors that inhibit the activity and/or spreading of *H. fraxineus* and, thus, promote dominance of other phytopathogenic species of fungi. The factors determining the low activity of *H. fraxineus* as well as the role of *Diplodia* spp. in the weakening of *F. excelsior* stands in and around Saint Petersburg require further research.

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