The Situation and Perspectives of European Ash (*Fraxinus excelsior*) in Ukraine: Focus on Eastern Border

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Abstract

The health condition of European ash (*Fraxinus excelsior*) stands in Ukraine has become worse since 2006. An alien invasive pathogenic fungus *Hymenoscyphus fraxineus* was firstly identified in 2011 in the eastern part of the country and subsequently, its presence had been confirmed in the western and central parts. The aims of our research were to evaluate the forest health of ash trees and identify the main causes of ash decline in different regions of Ukraine with emphasis on ash dieback and its association with collar rots.

Results showed that since 2013 the number of trees with ash dieback symptoms has been gradually increasing, reaching up to 92 % in 2018. Total mortality due to ash dieback was up to 9 % in 2018. Disease intensity remains high in the north and central Ukraine comparing with the east. Branch dieback, collar rots, epicormic shoots and bacterial disease of ash were more often in the eastern region, some symptoms were observed simultaneously. Ash bark beetle galleries, as well as foliage browsing insects, were found mostly in weakened and/or dying trees.

It was indicated that collar rots significantly increase the mortality of ash trees. *Armillaria* spp. fungi were found to be frequently associated with ash dieback on living stems and fallen trees in 2017, causing high rates of mortality in the northern and central regions. For further ash conservation and breeding programs, resistant trees in severely damaged regions should be selected to preserve genetic diversity in ash populations.

Keywords: *Fraxinus excelsior*, ash dieback, collar rot, bacterial disease, epicormic shoots, *Hymenoscyphus fraxineus*, *Armillaria* spp.

Introduction

A number of forest diseases, particularly invasive, may change forest woodlands and trigger biodiversity loss (Goberville et al. 2016). In Europe, the European ash (*Fraxinus excelsior* L.) is currently threatened across most of its distributional range by a new disease known as ash dieback, caused by *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya (Queloz et al. 2011) that is an emerging invasive fungus originating from Asian Far East (Baral et al. 2014). It has rapidly spread across Europe affecting mostly *Fraxinus excelsior* stands in many countries and causing massive ash decline (Pautasso et al. 2013, Gross et al. 2014). The health condition of ash stands in Ukraine has become worse since 2006 (Davydenko and Meshkova 2017). One of the main causes of decline for European ash (*Fraxinus excelsior*) was *H. fraxineus*, which was firstly found in Ukraine in 2010 (Davydenko et al. 2013), as well as other factors of ash damage were also acknowledged (Davydenko and Meshkova 2014, Goychuk and Kulbanska 2014).

The first appearance of symptoms of ash decline was observed in 2010 and the *H. fraxineus* was detected at low frequency in symptomatic shoots in 2012 (Davydenko et al. 2013). The ash decline varies significantly in different parts of Ukraine and it appears to be most serious in western and central Ukraine, where *H. fraxineus* was identified first in 2014 (Matsiakh and Kramarets

2014). So far, no symptoms of ash dieback have been observed in Ukraine on *Fraxinus pennsylvanica* Marsh and *F. angustifolia* Vahl. For ash dieback management, it is needed to get knowledge about ash dieback development and presence of associated diseases and pests in different regions. There is a good feasibility to find resistant or highly tolerant individuals in populations or in provenances as it has been reported for Danish, Swedish and German provenances (McKinney et al. 2011, Stener 2013, Metzler 2012, Enderle et al. 2015).

It has also been observed that collar rots increase decline process of ash dramatically and reduce the wood quality of the most valuable part of the stem. Moreover, collar rots and root rots decrease the stability of the trees resulting in tree hazards especially during windstorms. Collar rot can develop for a long time without any crown symptoms and wilting leaves being recognized (Langer 2017). Evaluation of forest health does not show decline process until visually healthy trees fall down.

Generally, collar rots are mostly caused by different fungal pathogens or wood decay fungi, as well as by *H. fraxineus* (Skovsgaard et. al. 2010, Enderle et al, 2017, Langer 2017), even though vulnerability to collar rot may be genetically determined (Langer 2017). Thus, it is very important to obtain such information in Ukraine including the development of collar rots and its interaction with ash dieback.

The aim of our research was to evaluate the forest health of ash trees and identify the main causes of ash decline in different regions of Ukraine with special emphasis on ash dieback and its association with collar rots and other pathogens.

Materials and Methods

Site conditions

A field study in Ukraine was carried out in the forest stands and forest shelter belts of 3 eastern, northern and western regions (Kharkiv, Sumy, and Zhytomyr respectively) located in the Forest Steppe (Kharkiv and Sumy) and Forest (Zhytomyr) zones (Figure 1). These were mixed forest stands between 20 and 80 years old with dominance of *Quercus robur* and *F. excelsior*. Permanent sampling plots were established in 2013-2016 (Table 1). Data on ADB before 2015 were obtained from our previous study (Davydenko and Meshkova 2017).

Methodology for evaluation of tree health

Our survey was based on annual assessment of forest health conditions. The survey report of tree damage symptoms and evaluation of ash dieback intensity was performed throughout all monitoring plots during the period of 2013–2018 in May and September. We have used the classification system that subdivides trees into

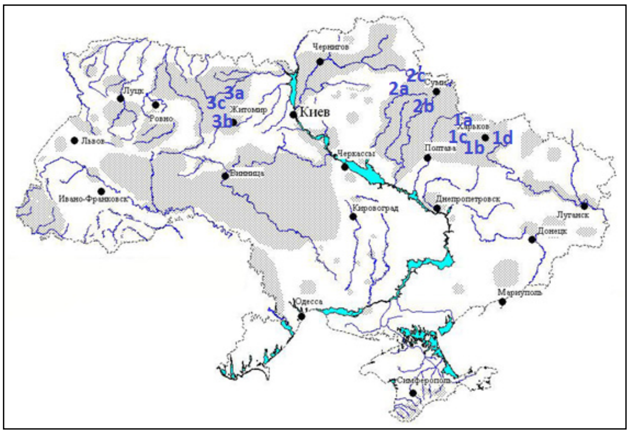

Figure 1. Map of Ukraine showing European ash distribution and sampling localities*
* All points on this map are an approximation of actual sampling localities, precise GPS coordinates are specified in Table 1:
1a, 1b, 1c, 1d – monitoring plots in the eastern region (Kharkiv) according to Table 1.
2a, 2b, 2c – monitoring plots in the northern region (Sumy) according to Table 1
3a, 3b, 3c – monitoring plots in the eastern region (Zhytomyr) according to Table 1

Table 1. List of monitoring stands of European ash used in this study

No plot	Name of locality	Type of stand	Age of trees	Coordinate
Eastern Ukraine, Kharkiv region, Zhovtneve Forestry				
1a	Pokotilovka	forest	30	49°55'31.7"N 36°10'28.8"E
1b	Vysokii	forest	25	49°53'48.3"N 36°14'04.9"E
1c	Babai	forest	40	49°53'12.4"N 36°08'39.6"E
Eastern Ukraine, Kharkiv region, Chuguevo-Babchanske Forestry				
1d	Kochetok	forest	80	49°53'48"N 36°44'14"E
Northern Ukraine, Sumy region, Krolevetske Forestry				
2a	Jaroslavets	forest	80	51°31'37.3"N 33°42'47.8"E
2b	Volokytyno	forest	48	51°28'18.2"N 33°46'40.7"E
2c	Zazerky	forest	55	51°28'25.4"N 33°39'10.0"E
Central Ukraine, Zhytomyr region, Emelchinske Forestry				
3a	Rudenka	forest	35	50°54'38.8"N 27°48'05.6"E
3b	Pidluby	forest	45	50°55'24.8"N 27°45'40.5"E
3c	Nykytyno	forest	47-50	50°53'01.8"N 27°45'06.1"E

six tree health classes (CMU 2016). These classes were determined for all trees assessed as follows: the 1st class – healthy; 2nd class – weakened; 3rd class – severely weakened; 4th class – drying up; 5th class – recently died; and 6th class – died over year ago by using such indicators as crown transparency, disease occurrence, crown dieback, and damage measurements of type, severity, and location (Table 2). All trees were assessed and assigned to one of the six classes. Index of tree condition for forest stand was calculated as a mean weighted value from the number of trees of each health category.

Ash dieback symptoms were evaluated separately using the following five-point system for disease intensity according to the number of symptomatic shoots and

Table 2. National scale used for rating of the forest health tree (condensed version)

Forest health class	Characteristics
1st class: healthy	Crown in good condition, no symptoms of damage. Leader and all lateral branches free of dieback symptoms, no foliage and stem damage
2nd class: weakened	Medium-density crown, current tree increment not less than ½ of average tree growth, minor mechanical stem or butt damages (up to ¼ stem perimeter); single epicormic shoots or mistletoe; canker or necrosis symptoms on stem or butt (up to ¼ stem perimeter); stem inclination up to 10% from the vertical axis; foliage diseases up to 75%; crown frost damage or defoliation by foliage browsing insects up to 25%
3rd class: severely weakened	Low-density crown, current tree increment is minor or absence; mechanical stem damage, canker and necrosis on stem or butt (up to ½ stem perimeter); many epicormic shoots or mistletoe; canker or necrosis symptoms on stem or butt (up to ½ stem perimeter); stem inclination up to 30% from the vertical axis; leader break not more than 2/3 of the living crown; foliage diseases up to 100%; crown frost damage or defoliation by foliage browsing insects up to 90%
4th class: drying up	Very low-density crown, yellowish and small-leaved foliage, crown dieback up to 2/3; absence of current tree increment; mechanical stem damage, canker and necrosis symptoms on stem or butt (more than 2/3 stem perimeter); numerous epicormic shoots; more than ½ shoots damaged by mistletoe; canker or necrosis symptoms on stem or butt (more than 2/3 stem perimeter); stem inclination more than 30% from the vertical axis; leader break more than 2/3 of the living crown; symptoms of damage by stem pests; fruit bodies of wood decay fungi; but some live foliage is still present.
5th class: recently died	Dead tree within one year
6th class: died over year ago	Dead tree more than a year ago

crown damage (Metzler et al. 2012): (0) no symptomatic shoots and ash dieback-symptoms; (1) more than 4 symptomatic shoots with necrotic lesions in 10 % of the crown; (2) more than 10 symptomatic shoots with necrotic lesions in 10–50 % of the crown; (3) more than 50 % of all shoots are symptomatic, and (4) dead trees.

By epicormic shoots, the trees were rated as follow: 0 – absence of epicormic shoots; 1 – sporadic epicormic shoots; 2 – multiple epicormic shoots; 3 – total stem coverage with epicormic shoots.

By bacterial cancer, the trees were rated as follows: 0 – absence of lens-shaped blisters and swellings; 1 – sporadic lens-shaped blisters and swellings, as well as vertical and lateral cracks; 2 – multiple lens-shaped blisters and swellings with numerous vertical and lateral cracks (Janse 1981).

Insects and fungi at the sample plots were identified by symptoms (defoliation, discolouration, necroses) and signs of damage (galleries, fungal fruiting bodies, spores). Apart from this, other biotic and abiotic damaging factors were recorded.

Detection of H. fraxineus in branches of F. excelsior

The observations and sample collecting focused on the presence of wilting leaves, branches with necroses and cankers on ash regeneration as well as on epicormic shoots of the tree stems from felled and fallen ash trees. All environmental samples with typical ADB symptoms were analyzed using morphological and molecular methods (Kowalski et al. 2015) and fungal cultures were isolated on Malt Extract Agar (MEA).

Examination of collar rots

In 2017, every sampled stem collar was examined for the presence or absence of stem necroses and *Armillaria* spp. in Zhytomyr, Sumy and Kharkiv regions. The percentage of affected collar circumference and the extent of decay were determined. Both the bark with white mycelium or rhizomorphs of *Armillaria* were sampled from all trees as well as all samples were collected from a necrosis where no *Armillaria* mycelium was visible. The fungi were isolated from collar lesions of each tree. For this, wood pieces were ground with metal beads and DNA was extracted using NucleoSpin® Plant II Midi kit (MACHEREY-NAGEL product) and PCR was done to identify *Armillaria* species using specific primers (Sicoli et al. 2003).

Pathogenicity test

A long-term assessment of the pathogenicity of *H. fraxineus* for Ukranian ash trees (*F. excelsior*) was carried out in Kharkiv region. Both sampling and isolation of *H. fraxineus* were performed according to Kowalski (2006). In May 2013, 25-35-years-old *F. excelrior* trees were inoculated with agar plugs with *H. fraxineus* by cutting out a piece (ca. 5×10 mm in size) of the bark on the stem ca. 120 cm above the root collar with a sterile scalpel, placing fungal inocula on the exposed sapwood and covering it up with the bark and wrapping with Parafilm™ sealing tape, as previously described (Kirisits et al. 2010). As a control, 10 ash trees of the same age were inoculated with sterile plugs of the same size of 3 % MEA.

Inoculated ashes were checked during the period 2014–2018 by measuring the necroses behind dying or dead bark that was removed from the stem. The size of each necrotic lesion was measured for estimation of its surface area (mm^2). Class of forest health condition, ash dieback score, degree of tree mortality and the size of necrotic lesions were evaluated in all variants during May and September each year. Re-isolation of inoculated fungus was attempted in 2016 and 2017 (Hauptman et al. 2016).

Statistical analyses

The differences in crown damage among plots of different sites and data of inoculation test were estimated using One-Way Analysis of Variance (one-way ANOVA) with a confidence level of 95 %. The Kruskal–Wallis tests were performed using the Mann–Whitney U tests with the Bonferroni correction as *post hoc* for variables). Tetrachoric factor analysis was computed to analyze correlation between the incidence of dieback and epicormic shoots using Microsoft Excel®. Remaining calculations were done using statistical software package PAST (i.e. Paleontological Statistics Software Package for Education and Data Analysis) (Hammer et al. 2001).

Results

Occurrence of ash damage symptoms in the eastern region

In total, 560 ash trees were assessed. The following eight types of ash damage were found in monitored ash stands during 2017-2018: frost cracks, mechanical damage of stems, epicormic shoots, branch dieback, bacterial disease and collar rot, wood decay fungi and insect feeding (foliage damage and galleries under the bark) (Meshkova and Borysova 2016). Branch dieback dominated with mean occurrence of 52 % and 85 % in 2017 and 2018, respectively (Figure 2). Visual symptoms of collar rots and epicormic shoots were also common on ash trees. The symptoms of collar rots were revealed in the ash trees of the 2nd – 5th classes of tree condition of forest health, more often for trees of the second - third classes of forest health tree condition (data not shown). Occurrence of foliage insects and bacterial disease was approximately the same for all the period of monitoring (Figure 2).

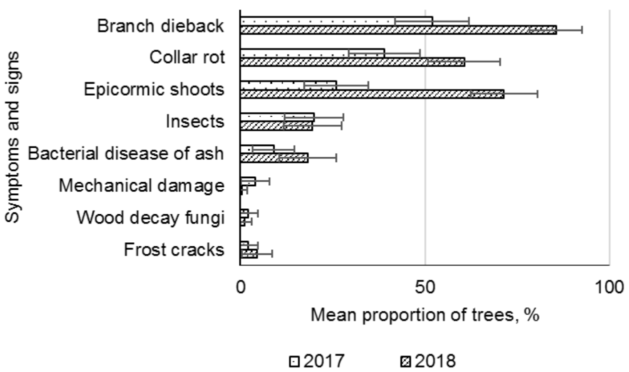

Figure 2. Proportion of European ash trees with different symptoms and signs of damage in 2017 and 2018, % (Kharkiv region, eastern Ukraine)

Among wood decay fungi, the fruit bodies of *Fomes fomentarius* (L.) Fr., *Ganoderma applanatum* (Pers.) Pat, *Laetiporus sulphureus* (Bull.) Murrill, *Bjerkandera adusta* (Willd.) P. Karst., *Oxyporus populinus* (Schumach.) Donk, and *Schizophyllum commune* Fr. were frequent at the all monitoring plots (Figure 2). The highest occurrence of fruit bodies was registered in the ash trees of the fourth class of forest health tree condition (data not shown).

About half of trees assessed had branch dieback of up to 10 % of the crown size. Among weakened trees and severely weakened trees the individuals with minor ash dieback symptoms (ADB score 1) dominated and approximately all dying up and dead trees had symptoms of ash dieback (Figure 3). Most weakened and severely weakened trees, as well as declining trees had sporadic or multiple epicormic shoots to a varying frequency (Figure 4).

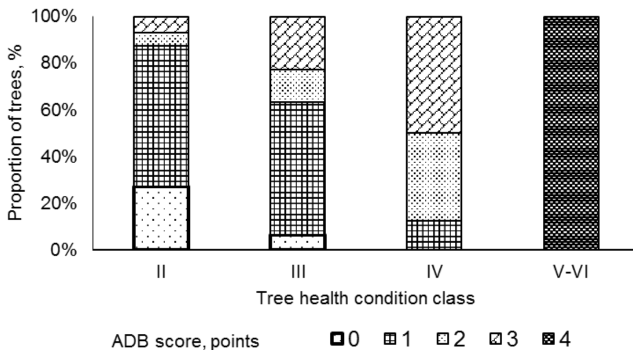

Figure 3. Distribution of European ash trees with different ash dieback (ADB) score* by tree health condition classes in 2018, (Kharkiv region, eastern Ukraine)**

* - ADB scores (ash dieback) symptoms were evaluated according to the following five-point system (Metzler et al. 2012): (0) no symptomatic shoots and ash dieback-symptoms; (1) more than 4 symptomatic shoots with necrotic lesions in 10 % of the crown;) (2) more than 10 symptomatic shoots with necrotic lesions in 10–50 % of the crown; (3) more than 50 % of all shoots are symptomatic and (4) dead trees.

** – trees of the 1st class were absent

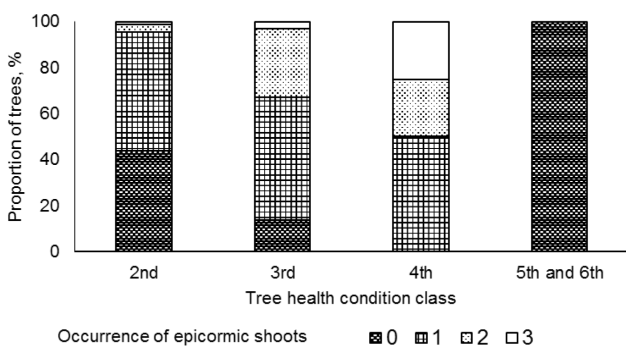

Figure 4. Distribution of European ash trees with different occurrence of epicormic shoots by tree health condition classes* in 2018 (Kharkiv region, eastern Ukraine)

0 – absence of epicormic shoots; 1 – sporadic epicormic shoots; 2 – multiple epicormic shoots;

3 – total stem coverage with epicormic shoots.

* - trees of the 1st class were absent

The highest proportion of trees with sporadic bacterial canker symptoms was revealed in the trees of the 3rd and 4th class of forest health (Figure 5). Both frost cracks and mechanical damage promote bark beetles and pathogens to penetrate under the bark.

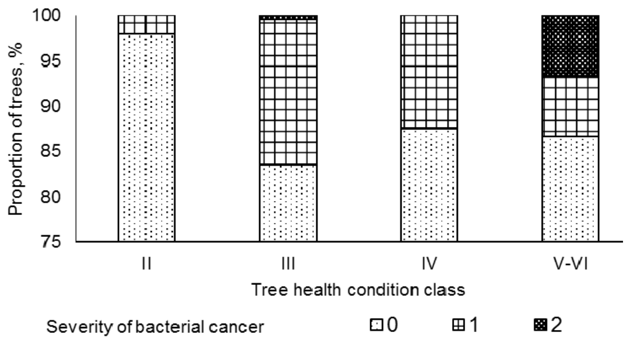

Figure 5. Distribution of European ash trees with different occurrence of bacterial cancer (tuberculosis) by tree health condition classes in 2018 (Kharkiv region, eastern Ukraine)* 0 – the absence of lens-shaped blisters and swellings; 1 – sporadic lens-shaped blisters and swellings as well as vertical and lateral cracks; 2 – multiple lens-shaped blisters and swellings with numerous vertical and lateral cracks (Janse 1981)
*- trees of the 1st class were absent

of living trees with mechanical or frost damage or in felled trees. The most common insects were *Hylesinus crenatus (*F., 1787), *Hylesinus fraxini* (Panzer, 1779), and *Hylesinus toranio* (Danthoine, 1788).

Many trees revealed several symptoms simultaneously (Table 3). Dieback and epicormic shoots occurred in 63.5 % trees, dieback and collar rot – in 49.9 % trees, epicormic shoots and collar rot – in 39 % trees. Combination of bacterial disease with mentioned symptoms occurred much less (7.4–9.5 % of trees). Statistical analysis (Table 3) showed strong positive correlation between incidence of dieback and epicormic shoots, moderate correlation between incidence of dieback and bacterial disease, as well as between bacterial disease and collar rot.

Development of ADB symptoms

In the monitoring period (2013 – 2018), occurrence of ADB incidence have changed from 1 % of the trees in 2013 up to 92 % in 2018, though intensity varied in different regions (Figure 6). The data of ADB intensity shown that about 90 % of trees were healthy or shown only occasional ADB symptoms (scores 0–1) in 2014 and the number of healthy trees remained only 10 % in 2018. About half of the trees (48 %) had serious ADB symptoms (score 2), whereas 30 % of the trees had more than 50 % of infested branches (class 3) or had already died

Table 3. Proportion of European ash trees with mixed damage and statistical analysis of correlation between mixed forms of damage in European ash trees (2018, Kharkiv region, eastern Ukraine) $\chi^2_{0.05} = 3.84$.

Damage type A	Damage type B	Proportion of ash trees with symptoms of mixed damage	Number of inspected trees with the damage: A and B	A without B	B without A	neither A nor B	N	r	χ^2 fact	Conclusion about correlation
Dieback	Epicormic shoots	63.5 ± 2.31	275	92	29	37	433	0.24	25.69	proven
Dieback	Bacterial disease	9.5 ± 1.41	41	326	2	64	433	0.10	4.15	proven
Dieback	Collar rot	49.9 ± 2.40	216	151	35	31	433	0.04	0.78	not proven
Epicormic shoots	Bacterial disease	7.4 ± 1.26	32	272	11	118	433	0.03	0.40	not proven
Epicormic shoots	Collar rot	39.0 ± 2.34	169	135	82	47	433	-0.07	2.36	not proven
Bacterial disease	Collar rot	7.6 ± 1.28	33	11	219	171	434	0.12	5.77	proven

Ash damage by foliage browsing insects from Geometridae and Tortricidae was not significant on the monitoring plots in 2017 and 2018 (on average about 20 %) and defoliation did not exceed 30 % (Figure 2). The most common foliage browsing insects were *Lytta (Lytta) vesicatoria (*L., 1758*)* (Coleoptera: Meloidae), *Tomostethus nigritus* (F., 1804) (Hymenoptera: Tenthredinidae) and *Macrophya (Pseudomacrophya) punctumalbum* (L., 1767) L. (Hymenoptera: Tenthredinidae). The ash weevil *Stereonychus fraxini* (DeGeer, 1775) (Curculionidae) was the most spread defoliator in some forest plots. Bark beetles galleries were found under the bark

(class 4) in 2018. Only in 2018, the total mortality due to ash dieback was up to 9 %.

Majority of symptoms and the highest rates of mortality were in the central (Zhytomyr) and northern (Sumy) parts of Ukraine, where symptoms were registered for up to 92 % of all trees and degree of mortality reached 10–12 % in 2018 (Table 4). Moreover, ADB intensity as determined by ADB class, in the northern and eastern regions has increased up to 3.7 and 3.2, respectively, while the severity of ADB in the central region has intensified only up to 1.16 over the last five years (Table 4). Nevertheless, the disease intensity remains

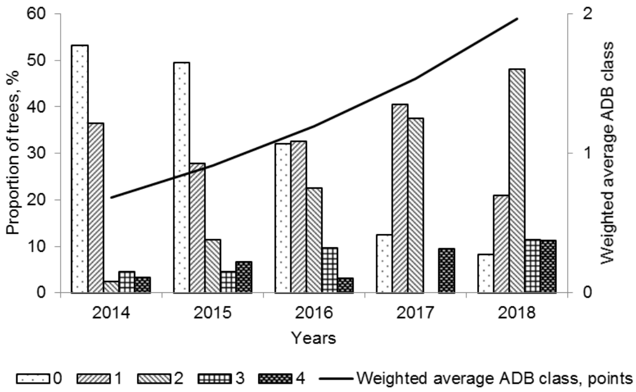

Figure 6. Proportion of ADB disease intensity* in all monitoring plots (Kharkiv, Sumy and Zhytomyr regions).
* Each tree was classified into one of five scores: (0) no symptomatic shoots and ash dieback-symptoms; (1) one or four symptomatic shoots, minor and indirect symptoms (uneven foliage expansion, necrotic lesions in healthy shoots; (2) more than 4 symptomatic shoots with necrotic lesions formation in 10 % of crown size; (3) more than 10 symptomatic shoots with necrotic lesions formation in 10–50 % of crown size; (4) more than 50 % of all shoots are symptomatic and (5) tree mortality

Table 4. The class of forest health condition and intensity of ash dieback (ADB) at the monitoring plots in different regions of Ukraine in 2013-2018

	ADB intensity, score*			Forest health condition, score**		
	Kharkiv (eastern)	Sumy (northern)	Zhytomyr (central)	Kharkiv (eastern)	Sumy (northern)	Zhytomyr (central)
2013	0.5	0.7	1.9	2.1	1.9	2.6
2014	1.3	1.2	3.0	2.3	2.0	3.0
2015	1.6	1.5	3.8	2.4	2.3	3.9
2016	1.3	1.0	1.5	2.4	2.2	3.8
2017	1.5	1.6	1.6	2.6	3.9	3.9
2018	1.6	2.6	2.2	2.8	2.2	4.1

* – ADB score – each European ash tree was classified into one of five score from 0 – no symptoms to score 4 as dead tree
** – forest health condition - each European ash tree was visually evaluated (crown density, dead branches, stem condition etc.) and classified into one of six score from 1 – healthy tree to 5 and 6 recently died and died over year ago tree

high in the northern (2.6 points) and western (3.2 points) regions comparing with the eastern region where by 2018 the intensity of damage by *H. fraxineus* has reached 1.6 points (Table 3).

At all sites, apothecia of *H. fraxineus* were found in each survey year. However, the amount of apothecia, and therefore, the infection pressure have differed between the regions. Numerous apothecia of *H. fraxineus* were observed in 2015–2017 in both the northern and western regions, where they were found in around 72 and 63 % monitored trees, respectively. In the eastern region, apothecia of *H. fraxineus* were found in forest litter only under 17–26 % of infected trees during annual autumn survey (from August to October).

Pathogenicity test

The health condition of ash trees inoculated with *H. fraxineus* has gradually declined over years starting from the point of inoculation. For the trees inoculated in 2013, the condition index of forest health changed from 1.39 in 2013 to 3.78 points in 2017 and then remained unchanged (Table 5). The highest level of tree mortality (27.8%) was over the first two years after inoculation (2015) and in the next year (2016) it has reached 38.9% (Table 5). Over the next two years, both ash dieback symptoms and condition index of forest health have increased; however, the rest of inoculated trees remained alive.

The Pearson correlation coefficient has shown a strong positive correlation between forest health condition index and ash dieback score over the monitoring period (Table 5).

Table 5. The interaction between ash dieback (ADB) intensity and forest health condition (FHC) index for European ash trees inoculated with *H. fraxineus* (2013–2018) for all monitoring plots

Date	Death of trees, cumulatively, %	ADB score*	FHC index**	Correlation	*p* value
May 2013	0	0	1.39	0.915	0.010
Sept 2013	0	0.06	1.78	0.914	0.004
May 2014	0	0.89	1.83	0.984	<0.001
Sept 2014	5.6	1.61	2.17	0.967	<0.001
May 2015	27.8	2.17	2.61	0.973	<0.001
Sept 2015	27.8	2.28	2.67	0.958	<0.001
May 2016	38.9	2.44	3.22	0.938	<0.001
Sept 2016	38.9	2.56	3.28	0.890	<0.001
May 2017	38.9	2.72	3.67	0.876	<0.001
Sept 2017	38.9	2.78	3.78	0.905	<0.001
May 2018	38.9	2.72	3.72	0.931	<0.001
Sept 2018	38.9	2.83	3.78	0.920	<0.001

* – ADB score – ash dieback intensity, each European ash tree was classified into one of five score from 0 – no symptoms to score 4 as dead tree
** – FHC index – forest health condition index, each European ash tree was visually evaluated and classified into one of six score according to Table 2; index of forest health condition was calculated as mean weighted value from trees number of each category of forest health condition.

For the inoculated trees, both the forest health index and ash dieback score demonstrated statistically significant increasing trend (Figure 6) over the experiment period (the Mann-Kendall trend test, Z=4.2615, *p* (no trend) = 2.03 E^{-05} for both parameters). The forest health condition of control trees did not worsen during our research and the forest health condition score did not exceed 1.3 points (data not shown).

Ash dieback symptoms developed almost at the same rate (Figure 7) except first two years after inocula-

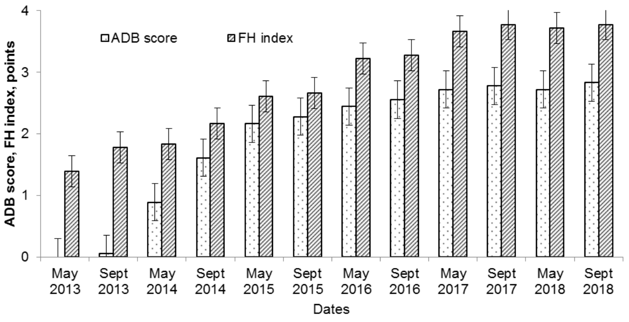

Figure 7. Dynamics of ash dieback intensity and forest health condition score for European ash trees inoculated with *H. fraxineus*

ADB score – ash dieback score*; FH index – forest health condition index**; bars mean standard error.

* Each tree was classified into one of five scores: (0) no symptomatic shoots and ash dieback-symptoms; (1) one or four symptomatic shoots, minor and indirect symptoms (uneven foliage expansion, necrotic lesions in healthy shoots; (2) more than 4 symptomatic shoots with necrotic lesions formation in 10 % of crown size; (3) more than 10 symptomatic shoots with necrotic lesions formation in 10–50 % of crown size; (4) more than 50 % of all shoots are symptomatic and (5) tree mortality.

** according to Table 2.

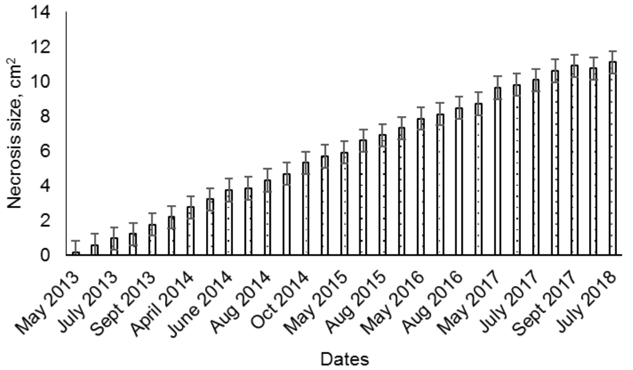

Figure 8. Development of necrotic lesions on European ash trees after inoculation with *H. fraxineus*

tion when ash dieback symptoms score increased up to 2.3 points in September 2016. Over the next two years, this index increased rather slowly and has reached 2.8 points in September of the 4th year after inoculation.

Re-isolations from inoculated trees were successful in 86.9% of attempted cases. Necrosis size ranged from 1.29 to 9.75 cm^2 with the mean value of 4.85 cm^2 for all the inoculated trees by the end of growing season of the 2018. Obtained results showed that there was significant difference in necrosis size between the compared groups as determined using the non-parametric Kruskal-Wallis tests (data not shown) as different factors may influence the necrosis size (Figure 8).

Development of crown symptoms of ADB and collar rot

The highest score of ash dieback symptoms and the number of dead trees were in the central and northern parts of Ukraine (Table 7). Moreover, substantial number of fallen ash trees was observed without any crown decline symptoms. Collar rot on fallen trees with numerous apothecia of *H. fraxineus* were found in the year 2017 survey. At each survey site in central and northern Ukraine, *Armillaria* rhizomorphs and collar rot were found frequently on bases of living stems and fallen trees in 2017 while only some trees with *Armillaria* rhizomorphs and no collar rot on bases of monitored living and dead trees were found in eastern Ukraine (Table 6).

The most common species, associated with collar rot were *Armillaria gallica* (94.0 %), *H. fraxineus* (92.3%), *Armillaria mellea* (73.8%) and *Armillaria* spp. (26.6 %).

Corresponding to the classes of ash dieback intensity, the proportion of trees with collar rot in Sumy region (northern Ukraine) was the highest for trees with score 1 and 2 (85.7 and 95 %, respectively) and all fallen dead trees were affected with collar rot (Table 6).

Much lower but still the high proportion of trees with collar rot in Zhytomyr region (central Ukraine) has been observed for ABD score 1 and 2 (80.9 and 68.8 %, respectively) and all fallen dead trees were affected with collar rot. Moreover, both *H. fraxineus* and *Armillaria* spp. were found to be associated with all trees with collar rot (Table 7). Only 25 % of trees with ADB score 1 and 2 were damaged only by *Armillaria* spp. in the northern region and 14.3 % and 6.3 % in the central region for ADB score 1 and 2, respectively (Table 6).

Table 6. Proportion of European ash trees affected with *Armillaria* spp. and *H. fraxineus* in 2017

	ADB intensity, score	The number of trees	Affected by			
			Collar rot, %	*Armillaria* spp., %	ADB, %	*Armillaria* +ADB, %
Sumy (north)*	0	2	0	0	0	0
	1	14	85.7	25	0	58.3
	2	20	95	25	25	60
	3	0	0	0	0	0
	4	6	100	0	0	100
Zhytomyr (centre)*	0	0	0	0	0	0
	1	21	80.9	14.3	0	80.9
	2	16	68.8	6.3	6.3	68.8
	3	0	0	0	0	0
	4	6	100	0	0	100
Kharkiv (east)	0	0	0	0	0	0
	1	23	0	0	95.6	4.3
	2	19	0	0	94.7	5.2
	3	0	0	0	0	0
	4	0	0	0	0	0

* – numerous apothecia of *H. fraxineus* were found in survey year (2017).

Discussion

Epidemics caused by invasive forest diseases, including ash dieback in Europe, are a great problem for all European countries, causing the loss of forest trees and compromising food security, biodiversity, and national economies (Harper et al. 2016). The common ash trees (*Fraxinus excelsior*) that are damaged by the *H. fraxineus*, represent a great loss on a continental scale. Therefore, ash dieback management and practical recommendations should be based on experience of European countries, where the disease has been established for more than a decade (Poland, Lithuania, Sweden, Germany, etc). Revealing this fungus in the ash stands of Ukraine can lead to decrease in the number of ash trees in the forest stands and even to its extinction (Davydenko and Meshkova 2014).

In agreement, our study demonstrates that the majority of monitored ash trees have shown the gradual increase of ash dieback symptoms and the significant decreasing of forest health condition since the start of our experiment and measurements in 2013.

Since 2010, ash dieback has extended across Ukraine and it has become a serious problem in the Ukrainian forest and urbanised zones, where the causative fungus has invaded the area rapidly but with varying intensity of damage. Our results also indicate significant differences in intensity of ash dieback in various regions of Ukraine while being more pronounced in the northern and central regions that are characterized by higher levels of precipitations as compared to the eastern region, where the vegetative season is dry and hot (Davydenko and Meshkova 2017). Moreover, no ADB symptoms were registered in the southern areas (personal observations). According to our personal observations, the ash dieback is the more intensive for young trees and epicormic shoots, developing after clear-cuts, but it seems to be also spread to all ages. Therefore, recommendations for disease management concerning mitigation of consequences of ash dieback in the forest stands, nurseries and urban zone should be done.

However, infection pressure may have differed in intensity between the locations as exemplified by many trees that have minor and medium symptoms (health index scores 1–2). On the other hand, a large proportion of trees was affected by a combination of different diseases (*Armillaria gallica* or *Armillaria mellea* and *H. fraxineus*) which have likely deteriorated the overall health condition of ash trees in the study areas (Table 5). One of these agents, *Armillaria mellea* is an aggressive pathogen, which causes white root rot in a wide range of hosts (Guillaumin et al. 1993). *Armillaria* root rot were identified on dead and dying ash trees as well as on the numerous hardwood trees of other species, indicating that *Armillaria* sp. is responsible for manifestation of the disease. Such saprotrophic species as *Armillaria gallica* was also present in most collar rot on ash trees. There was complete overlap of both *A. mellea* and *A. gallica* with *H. fraxineus* (data not shown), suggesting that *Armillaria* fungi has likely benefited from the infections of *H. fraxineus* and consequent weakening of the trees. By contrast, the *A. gallica* and *A. mellea* overlapped only rarely (data not shown), suggesting similar colonization strategies.

The results of the present study also demonstrate that *H. fraxineus* is likely to continue expansion and establishment in other regions of Ukraine and thus can be considered as an important pathogen of *F. excelsior* and could affect ash seedlings (Davydenko and Meshkova 2017) and older trees, particularly with *Armillaria* root rot, within a short period. It appears that ash dieback along and with other pests and pathogens plays a crucial role in the massive of ash decline in Ukraine as it was previously confirmed in other studies (Gross et al. 2014, Enderle et al. 2015) and situation with tree health can be expected to be worse in the future (Davydenko and Meshkova 2014, 2017).

In Ukraine, despite the disease presence for at least 10 years, numerous *F. excelsior* trees have remained vigorous, without any visible ash dieback symptoms. Moreover, a part of inoculated ash trees remained alive and necrosis size did not change significantly over two years although presence of *H. fraxineus* has been confirmed using specific primers. Some trees also showed low levels of symptoms even when neighbouring trees were severely damaged and numerous apothecia of *H. fraxineus* were found in damaged trees. In the present study, the inoculation of ash trees demonstrated a formation of different necrosis size, while dead trees showed the longest lesions (data not shown). The inoculation was performed in the eastern region where unfavourable factors (dry and hot vegetation period) for diseases, particularly ash dieback and collar rot, were registered over the experiment. In our assessment, the trees with the smallest necrosis and with suppressed necrosis development were considered as more or less tolerant to the pathogen at this stage. The observed variation in the susceptibility to ADB provides the prospect for greater utilization of breeding for resistance with the aim to manage this disease in the future. However, the significant differences in the tolerance after inoculation suggest that further studies are needed to obtain conclusive results. Therefore, it is recommended that visually healthy (resistant) ash tree should be selected (McKinney et al. 2014) to initiate further breeding programme and for the conservation of the species.

Conclusion

The ash dieback pathogen spread over the whole country except the southern regions over six years. Since 2013, the number of infested trees has increased every year, reaching up to 92 % of affected trees in 2018 as compared to 1 % in 2013. However, the intensity of ash dieback was different in another regions. Within five years, various studies allowed to evaluate the disease intensity and the impact of other abiotic and biotic factors. Our study demonstrated an increase in ash dieback severity, as well as tree mortality caused by collar rots. Moreover, the occurrence of bacterial canker and epicormic shoots has also increased in the eastern region in recent years. The high damage to ash stands caused by both *H. fraxineus* and *Armillaria* spp. leads to significant mortality in the northern and central regions and the decrease of survival of potentially resistant trees. Moreover, the significant increase in the number of trees associated with pathogens raise new questions concerning the future impact of ash dieback and collar rots that is not yet statistically predictable. For this reason, potentially resistant trees in severely damaged regions should be selected for further conservation and to preserve genetic diversity in ash populations.

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