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EUROPEAN ASH (FRAXINUS EXCELSIOR) DIEBACK – SITUATION IN EUROPE AND UKRAINE

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Publications, mainly for 2013–2014, and authors' own data on ash dieback, caused by Hymenoscyphus fraxineus (anamorph Chalara fraxinea) are reviewed. Disease is known now in over 25 countries of Europe, since 2010 – in Ukraine. The presence of pathogen in Kharkiv, Sumy, Poltava, Kyiv and Zhytomyr regions is proved by molecular methods. The main symptoms of disease are: quick successive crown dieback, necrotic spots on the bark of shoots, discoloration of wood and leaves, leaf necrosis, premature leaf-shedding, stem necroses. Investigations are directed on study of pathogen development, virulence of certain fungi isolates, susceptibility of certain species and populations of Fraxinus sp. to infection, as well as on development the guidelines for prevention of negative consequences of disease spread for forest management and forest ecosystems.

Key words: *Fraxinus sp., ash dieback, Hymenoscyphus faxineus (Chalara fraxinea), guidelines for forest management.*

Introduction. European ash (*Fraxinus excelsior* L.) is the most common species of this genera in temperate Europe [42], including Ukraine [8]. Ash dieback caused by *Hymenoscyphus fraxineus* (anamorph *Chalara fraxinea*) is a disease that has emerged in North and Central Europe in the 1990s [12]. This disease was first observed in Poland and it has rapidly expanded over most part of European ash area in Europe [34, 42, 49]. Such stepwise pattern of geographic spread in many directions is characteristic feature for alien invasive species.

Ash dieback caused by *H. fraxineus* was firstly found in forest stands and forest shelter belts in the eastern Ukraine in 2011 [17], but perhaps first symptoms appeared more earlier in western Ukraine which borders Poland where the disease was reported first.

Before 2010, there was little concern regarding ash health condition in Ukraine, as no dieback symptoms were reported [1, 39]. However, a lot of morphological symptoms of ash dieback were observed in different parts of the Ukraine during 2010–2014. Such symptoms include quick successive crown dieback, necrotic spots on the bark of shoots, discoloration of wood and leaves, leaf necroses, premature leaf-shedding, stem necroses etc [30, 31, 36, 45].

Presence of ash dieback pathogen in forest stands and shelter belts of the eastern Ukraine was firstly proved by molecular methods in 2011 [17]. However, it could get some years earlier to the west regions of Ukraine, which border with Poland, where disease was described firstly [33]. Notwithstanding well-known and validated dates about distribution of the ash dieback in Western Europe, little information is known about status of this disease from Eastern and South-Eastern European countries, except some personal communications and publications [5, 17].

The aim of our research was the review of publications and our own data on ash dieback in Ukraine and in Europe, summarising of practical guidelines for forest management from different European countries with long ash dieback experience, and identification of our future researches. Also, we have examined the pests and fungi associated with ash trees in Ukraine.

Materials and methods. *The literature review* was carried out using databases of Web of Science and FRAXBACK website [48] with relevant links. The FRAXBACK action is funded by the European Cooperation in Science and Technology (COST) for a 4 year period starting May 2012. The main objective of the Action is to generate comprehensive understanding of *Fraxinus* dieback phenomenon, and to elaborate state of the art practical guidelines for sustainable management of *Fraxinus* in Europe.

Field researches in Ukraine were carried out in 2010–2013 in the forest stands and shelter belts of Kharkiv, Sumy, Poltava, Luhansk, Kyiv and Zhytomyr regions. These stands were 10 to 80 years old with dominance of *Quercus robur* and *F. excelsior.*

Sanitary condition of ash trees was assessed in the sample plots. Six categories of sanitary condition were used according to "Sanitary rules in the forests of Ukraine" [9] (I – healthy; II – weakened; III – strongly weakened; IV – drying; V – fresh dead; VI – old dead). Also, for each site visually condition of trees have been evaluated according to scale of possible symptoms for ash dieback [38], where 0 – no *Chalara*-symptoms, 1 – minor and indirect symptoms (uneven foliage expansion, necrotic lesions in healthy shoots and dead branches in 10 % of crown size; 2 – dead shoots and branches with necrotic lesions formation in 10–50 % of crown size; 3 – death of 50 % of crown size, wood discoloration, necrotic lesions, dead leaves, shoots, branches, 4 – total crown damage, necrotic lesions on green shoots, branches, stem and butt.

Insects and fungi in the sample plots were identified by symptoms (defoliation, discoloration, necrotic lesions) and signs (galleries, fungal fruiting bodies, spores). Symptomatic leaves and branches (i. e. having necrotic lesions) were cut and individually packed into plastic bags.

In the laboratory, collected material was sorted by necrotic lesions of bark and leaves. To 5–10 pieces (by 5 × 5 mm) were cut from each sample of leaf or twig. The surface was sterilized with ethanol. These samples were used for fungal culturing on MEA (3 % malt extract agar) as well as for DNA extraction and direct sequencing to check the presence or absence of *H. fraxineus* using PCR-based techniques and species-specific primers [27, 29].

Results. European ash (*F. excelsior*) is one of 43 *Fraxinus* species occurring in temperate and subtropical regions of the northern hemisphere [42]. This species is tolerant to soil fertility, drought and frost, grows in mixed and pure forests and urban stands, shelter belts, arboretums and is of considerable socio-economical importance, particularly in Ukraine [2, 8].

Green ash (*F. lanceolata* Borkh = *F. viridis* Michx.) is also spread in the forest stands and shelter belts of the southern and eastern parts of Ukraine. Another ash species *F. pennsylvanica* Marsh. (= *F. pubescens* L.) is fast-growing and frost tolerant, but is rather rare [1, 8, 39].

Area of ash forest stands is 151.6 thous. ha, that is 2.4 % of forest covered area of the State Committee of Forest Resources of Ukraine. For last 10 years this area has increased by 34.4 thous. ha (29.8 %). Stock of ash stands is 212.4 m^3/ha, the mean age is 62 years old [2].

Sanitary condition of ash stands in Ukraine has become worse since 2006, when area of drying stands has exceeded 3.4 thousand ha [4]. Declining ash stands were registered in different natural zones of Ukraine. For example, 817.1 ha of ash stands have declined in Vinnitsa region, 659 ha in Cherkassy region, 764.3 ha in Mykolaiv region, 495.7 ha in Kherson region, 212.9 ha in Luhansk region. In considerable degree it is explained by decrease of annual precipitation. This index in Kharkov region was equal to 616.1 mm in 2005, and in 2006, 2007 and 2008 it was equal to 524.5, 522.7 and 475.8 mm respectively. The next three years annual precipitation was about 600 mm, but in 2012 decreased to 465.3 mm.

For 2005–2013 sanitary condition of ash trees has revealed the trend to worsening (in average from I.7 to III.3 points). In the depth of forest stands sanitary condition of ash was the most stable (I.7–II.3 points). The most dramatic decrease of ash sanitary condition was registered on the border with clear-cuts (from I.9 to IV.3 points), where drying trees had been observed in 2008, and dead ones in 2010. In the plot, where sanitary felling was carried out in 2007, sanitary condition of ash changed from I.7 to III.3 points. Such process was more dramatic near the western side of clear-cut (from I.3 to III.3), than near the southern side (II – II.5), and the most sharp decline was registered since 2009. Obtained data are explained by peculiarities of microclimate change after felling, as well as with appearance of wounds on stems during it [39].

Bark beetles *Hylesinus crenatus* (Fabricius, 1787) (= *H. prutenskyi* Sokolovskii, 1959) and *Hylesinus varius* (Fabricius, 1775) (= *H. fraxini* (Panzer,

1799) = *H. orni* Fuchs, 1906 = *Leperisinus varius* (Fabricius 1775) were found in the stems and branches of weakened ash trees [6].

Many species of foliage browsing insects were found in the crowns of ash but the most of them prefered oak foliage for feeding. The Spanish fly *Lytta vesicatoria* was found in 2012, and sawflies *Tomostethus nigritus* F. and *Macrophya* (*Pseudomacrophya*) *punctum-album* L. considerably damaged ash in 2002 and 2012–2014, especially in urban stands and lighted forest stands and shelter belts [7]. After spring defoliation the crowns of ash were partly recovered during the summer.

However, quick spread of ash dieback across the European countries makes conjecture about possibility to change ash stands condition dramatically in the nearest future. In eastern Ukraine, the emergence of first symptoms of ash decline in 2010 and occasional detection of *H. fraxineus* in symptomatic shoots (5.6%) suggested that this disease was at an initial phase of its spread in local stands of *F. excelsior* [17]. Also, ash dieback in pure and mixed tree stands has been observed in Western Ukraine, i.e. from the area which borders with Poland where the disease outbreak has started [5].

In the West Podolia tuberculosis (pathogen – *Pseudomonas syringae pv. savastanoi* (Smith 1908) Young et. Al. 1978)) is reported to be the important cause of ash stands decline. This pathogen affects stems, branches, twigs and buds of European Ash [1]. In the same region, *Ligniodes enucleator* Panz., *Dasineura fraxini* Kieff., *Prociphilus nidificus* Loew., *Fonscolombea fraxini* (Kalt.), *Psyllopsis fraxini* L., *Tortrix convayana* F. and *Pseudargyrotoza conwayana* F. are spread [1] and can play certain role in the vectoring the pathogens of ash during feeding on vegetative and generative organs of tree.

The main symptoms of ash decline in Ukraine are uneven flushing, leaves, stem and shoot necroses, discoloration of wood and premature leaf-shedding. Dieback of common ash caused by *H. fraxineus* was proved using molecular methods for samples from Sumy, Poltava, Kharkov, Kiyv and Zhytomir regions [3, 17] and by morphological signs for Lviv, Ivano-Frankivsk and Ternopil regions [5].

We present the main results of investigations on *H. fraxineus* and ash dieback including our own researches.

Taxonomy of Hymenosyphus fraxineus. Disease-causing agent of ash dieback was described by T. Kowalski in 2006 [34] as a new fungal species, *Chalara fraxinea*). In 2009, based on morphological and DNA sequence comparisons, *C. fraxinea* was suggested to be the asexual stage of the ascomycete *Hymenoscyphus albidus*. This was a major surprise, as *H. albidus* is a widespread common saprotroph species well-known across Europe since 19th century as harmless decomposer of shed ash leaves. *H. albidus* has never been reported to be pathogenic [12, 13]. However, in 2010 more detailed molecular investigations using ISSR-PCR amplification led to conclusion that the disease agent is not *H. albidus*, and the ash dieback pathogen was described as *H. pseudoalbidus* – a species entirely new to science [] (Queloz et al., 2011). The both species are morphologically very similar or indistinguishable. In 2012, *H. pseudoalbidus* was shown to be identical to *Lambertella albida*, which was found on petioles of *F. mandshurica* in Japan and described by Hosoya et al. in 1993 [50]. *L. albida* is a weak pathogen, while *C. fraxinea* dominates in fungal complexes in the declined ash stands [29, 43]. In 2014, in accordance with rules of fungi nomenclature, specific epithet in *Hymenoscyphus* was changed from "*pseudoalbidus*" to "*fraxineus*" [13].

Symptoms. According to majority scientific papers and pictures the typical symptoms of ash dieback become obvious on the leaves within months of infection. We can observe necrotic lesions on leaves, petioles and shoots up to wilting and dieback of twigs and shoots. Inner-bark lesions are characterized by a typical wood discoloration and emergence of dark brown smooth lesions on stem and branches. After top shoot dieback, its apical dominance becomes broken [11, 14, 31, 47]. Young ash trees usually die after stem infection. The growth of new shoots in still living parts of the tree was observed as substitution of dead branches and shoots [6]. Many researchers record a bushy mix of dead and living shoots [14, 37, 30].

Distribution in Europe. Thus, dieback of *F. excelsior* has firstly been observed in early 1990s in north-eastern Poland [34] and Lithuania [12]. In year 2002 the

disease had only been observed locally in southern Sweden but in 2004 it spread throughout whole Sweden, towards western and central parts to neighbouring isles of Finland and Denmark [12, 32, 44]. In year 2002, the disease was for the first time recorded in the north-eastern Germany, Czech Republic and Slovakia [28], and in 2005 in Austria [24]. Subsequently, in year 2007 ash dieback occurred in Hungary [46] and Slovenia [41]. In 2007 – 2008 it has emerged in Norway [49], Switzerland [22], France [15], and in 2009 it was observed in Italy and Croatia [49], in 2010 in Belgium, Netherlands and Ukraine [17, 26, 49]. In 2012, the pathogen was also detected in UK tree nurseries [10, 42].

In total, ash dieback caused by *H. fraxineus* was reported from over 25 European countries [10]. This fungus has supplanted the harmless decomposer *H. albidus*, because the both species occupy the same ecological niche [20, 38].

Distribution in Ukraine. In eastern Ukraine (in Kharkov region), characteristic symptoms of ash dieback were noted for the first time in 2010, as a sparse crowns, bark necroses and wood discoloration in shoots (observation by R. Vasaitis). It means that the trees were infected as far back as in 2009. The presence of *H. fraxineus* in symptomatic ash shoots was supported by molecular methods later [17]. It was assumed that pathogen could be present in other regions of Ukraine, especially in western regions, which border with Poland where the disease outbreak has begun. For this, 240 wood samples of ash symptomatic twigs and 24 pure cultures were used for DNA extraction. In total, three set of *C. fraxinea*-specific primers were used. So, during 2012–2013 we have confirmed the presence of *H. fraxineus* in the samples from five regions of Ukraine (Table 1). Although ash dieback symptoms were registered in many sample plots, severe mortality of ash was not observed. The trees with 10 to 50 % of dead shoots and branches (2 points) with necrotic lesions were found as often as dead shoots and branches without such symptoms (Table 1).

Absence of *H. fraxineus* in symptomatic shoots and leaves from Luhansk region can be related with high temperature (up to 45°C) and low precipitation of vegetative season of 2013, which were unfavorable for pathogen.

1. Occurrence of *H. fraxineus* in the samples of European ash from six regions of Ukraine

Site of sampling	Number of samples	Samples with necroses	*H. fraxineus* presence/ absence	Sanitary condition of tree	Symptoms of disease evaluation [37]
Sumy region					
Trostyanets	6	leaves	presence	2	2
Trostyanets	6	wood	presence	2	2
Trostyanets	35	wood	presence	2	2
Sumy	9	leaves	absence	2	2
Sumy	9	wood	absence	2	2
Sumy	34	wood	presence	4	2
Ohtyrka	7	leaves	presence	4	2
Ohtyrka	7	wood	presence	4	2
Shostka	15	wood	absence	2	2
Shostka	16	wood	presence	3	2
Shostka	17	wood	absence	2	2
Shostka	18	wood	presence	3	2
Shostka	19	wood	absence	2	2
Shostka	20	wood	presence	2	2
Kharkiv region					
Izum	1	leaves	presence	2	2
Izum	1	wood	presence	2	2
Izum	36	wood	presence	3	2
Pokotilovka	2	leaves	absence	3	2
Pokotilovka	2	wood	absence	3	2
Pokotilovka	3	leaves	presence	3	2
Pokotilovka	3	wood	presence	3	2
Pokotilovka	4	wood	presence	3	2
Pokotilovka	5	wood	presence	3	2
Pokotilovka	8	wood	absence	4	3
Zmiyv	21	wood	presence	2	2
Zmiyv	22	wood	presence	2	2
Molodeznii park	23	wood	absence	3	2
Molodeznii park	24	wood	absence	3	2
Krasnograd	25	wood	presence	3	2
Krasnograd	26	wood	presence	3	2
Poltava region					
Kopyly	10	leaves	presence	2	1
Kopyly	10	wood	absence	2	1
Kopyly	11	leaves	absence	2	1
Kopyly	11	wood	presence	2	1
Kyiv region					
Boryspil	12	leaves	presence	2	2
Boryspil	12	wood	absence	2	2
Kiev	13	leaves	presence	2	1
Kiev	13	wood	absence	2	1
Zhytomyr region					
Novograd-Volynskii	14	leaves	presence	2	3
Novograd-Volynskii	14	wood	presence	2	3

Table continuation

Site of sampling	Number of samples	Tree tissues	*H. fraxineus* presence/ absence	Sanitary condition of tree	Symptoms of disease evaluation [37]
Luhansk region					
Svatovo	27	wood	absence	3	2
Svatovo	28	wood	absence	3	2
Severodonetsk	29	wood	absence	3	2
Severodonetsk	30	wood	absence	3	2
Severodonetsk	31	wood	absence	3	2
Schastie	32	wood	absence	4	1
Schastie	33	wood	absence	3	1

Obtained data prove the spread of *H. fraxineus* in the territory of Ukraine from west to east, except southern regions, where we plan to carry out respective investigations. However, the spread of ash decline into eastern Ukraine is relatively slow [5, 17].

Life cycle of H. fraxineus (Fig. 1 below is reproduces from [23]). Ash dieback fungal pathogen spreads during summer (June-August in mainland Europe) with air-borne ascospores produced on infected fallen leaves. These ascospores infect healthy ash trees through green leaves during summer [14, 20, 33, 49]. This results in withering and dying of leaves, and shoot lesions and stem lesions development as the fungus grows into and through the leaf and into the woody tissue, gradually decline of branches and stem [20, 35]. *Chalara fraxinea* – the anamorph (asexual) stage of *H. fraxineus* develops during autumn and overwinters in the ash litter in leaf rachises from previous year [14, 20, 30, 33, 49]. Infected leaves fall to the ground, and pathogen forms black pseudosclerotia in the petioles to survive in adverse conditions. If two mating types of the fungus are present within the infected petioles, the fungus undergoes sexual reproduction and the following summer produces spore-bearing apothecia [14, 20]. The main time of sporulation is on June to early September [14, 20, 49], however in the suitable conditions it starts earlier and completed in October [20].

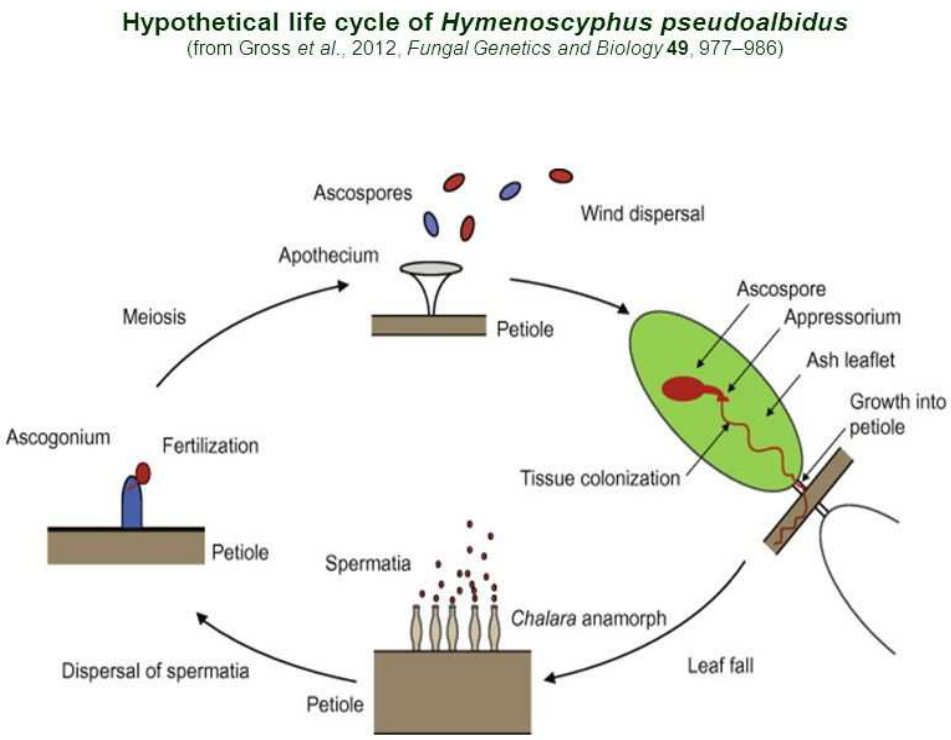

Fig. 1. Hypothetical life cycle of *H. fraxineus* (reproduced from [23])

Usually, *H. fraxineus* is quite resistant to dry hot conditions [21, 23]. In adverse conditions the fungus sometimes delays production of apothecia, but survives for at least two years, producing apothecia in the subsequent summer [21]. However, we have not revealed any apothecia on the leaf rachises during our sampling in Ukraine in 2010–2011, which can be related with unfavourable weather for production the fruit bodies [17]. Otherwise, much fruit bodies were collected in the same area in 2013–2014. Also, sampled petioles without any fructification from the same dry plots produced mature apothecia in the moist chamber in laboratory. It confirms the importance of rather high air humidity for development of normal apothecia [23]. In accordance with data of Swiss researchers [21] new apothecia can be formed on the pseudosclerotia throughout second or even third year after abscission thereafter the entire life cycle is completed.

Methods for pathogen identification. The ash tissue dies directly after pathogen entry and *H. fraxinus* is classified as a necrotroph [16].

Recently died shoots, leaves, petioles with typical symptoms of ash dieback can be used for correct identification of pathogen. *H. fraxineus* is identified with method of pure cultures or PCR-based methods using specie-specific primers [22, 27, 29] or sequencing.

Fungal cultivation in the laboratory is carried put on 3 % malt-extract agar with addition of fresh or frozen ash leaves. Pure cultures store in darkness at 4°C [27, 29].

Discussion and practical recommendations. Life cycle and pathogenicity of *H. fraxineus* under field condition are studied well [20, 30, 42]. Experience from European countries (Lithuania, Sweden, Swiss, Germany), where the disease has been established for more than a decade, as well as revealing this fungus in the ash stands of Ukraine, show, that spread of ash dieback can bring to decrease of the part of ash trees in the forest stands and even to its extinction. Therefore we summarize the practice experience and recommendations for disease management concerning mitigation of consequences of ash dieback in the forest stands, nurseries as well as artificial regeneration.

Practical recommendations for disease management. So far as *H. fraxineus* is an invasive pathogen, which damages *F. excelsior* and other *Fraxinus* species [18, 30, 47, 49], the main practical recommendations are common for invasive pathogens:

– maintaining diversity of species associated with ash;

– decrease the spread of ash dieback;

– maintaining genetic diversity in ash trees with the aim of ensuring the presence of ash in the long term.

The main recommendations of Forestry Commission UK [19] and Sweden and German researchers [32, 40] regard forest management in ash stands, where ash dieback can spread. In line with this, forest management measures should be directed on growing healthy and quite resistant stands. It will be effective in retardation of disease development, but will not be able to stop its spread.

Selective felling is effective at relatively low development of disease. The trees with damage over 50 % of crown size and presence of epicormic shoots must be cut, to prevent the formation large amount of spores for infection susceptible trees. The

trees with damage up to 50 % of crown size must be regularly inspected taking into account also spread of other pathogens (particularly *Armillaria* sp.).

Infected urban or veteran ash trees must not be felled, because they can provide many important environmental and social benefits. These trees give the possibility to maintain biodiversity of species (invertebrates, lichens etc.) associated with ash in the green zones of settlements. Pruning of damaged and dead branches and twigs will help to maintain ornamentality of these trees.

Attention must be paid to increase of genetic and age diversity of forest stands, as well as abstain from planting new ash stands, because young the saplings are the most susceptible to dieback. Use of fungicides for protection of seedlings does not ensure long-term effect, and the pathogen quickly propagates in young trees. Taking into account the data on heritability of resistance to *H. fraxineus*, it is recommended to promote development of natural regeneration from seeds of resistant trees, which survive in the focus of disease [19].

In the stands with dieback over 50 % ash trees, both planting and promotion to natural regeneration of ash are not recommended [40]. At the same time, one can assume that due to genetic biodiversity of ash populations, the most resistant trees with high reproduction ability will survive.

Conclusions. 1. Recent worsening of sanitary condition of European ash (*Fraxinus excelsior*) in Ukraine is connected by influence of assemblage of biotic factors (pests, pathogens), abiotic factors (weather conditions) and anthropogenic factors (particularly, forest management).

2. Ash dieback (pathogen – *Hymenoscyphus fraxineus*) is the main cause of weakening of ash stands in over 25 countries of Europe. Cycle of development of this pathogen and peculiarities of its spread in Europe has been investigated. Recommendations for disease management concerning mitigation of consequences of ash dieback in the forest stands, nurseries as well as artificial regeneration have been developed.

3. In Ukraine ash dieback pathogen was found in 2010 in the forest stands and forest shelter belts of Kharkov region, later in Sumy, Poltava, Kyiv and Zhytomyr

regions, which is proved by molecular methods. The main symptoms of disease include quick successive crown dieback, necrotic spots on the bark of shoots, discoloration of wood and leaves, leaf necrosis, premature leaf-shedding, stem necrotic lesions.

4. Ash dieback spreads rather slowly in the territory of Ukraine, especially in the east part of it. Crown damage does not exceed 10 – 50 %, noticeable mortality is registered only among trees below 15 years old.

5. Experience of European countries on threat of ash dieback show, that it is necessary to pay attention to monitoring the spread of disease in Ukraine and investigation the peculiarities of susceptibility of different populations and genotypes of European ash to *Hymenoscyphus fraxineus*.

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Наведено огляд публікацій переважно 2013–2014 рр. та власних даних стосовно хвороби відмирання ясена, яка спричиняється грибом Hymenoscyphus fraxineus (анаморфа Chalara fraxinea). Хвороба нині відома у понад 25 країнах Європи, з 2010 року – в Україні. Молекулярними методами підтверджено наявність збудника хвороби у Харківській, Сумській, Полтавській, Київській і Житомирській областях. Основними симптомами хвороби є поступове відмирання крон, некротичні плями на корі пагонів, знебарвлення деревини та листя, некрози листя, передчасне опадання листя, некрози стовбура. Дослідження спрямовані на вивчення циклу розвитку збудника хвороби, вірулентності окремих штамів, сприйнятливості окремих видів і популяцій роду Fraxinus sp. до інфекції, а також на розробку рекомендацій щодо запобігання негативним наслідкам поширення хвороби для лісового господарства та лісових екосистем.

***Ключові слова**: ясен Fraxinus sp., хвороба відмирання ясена, збудник Hymenoscyphus faxineus (Chalara fraxinea), симптоми відмирання ясена, поширеність і розвиток відмирання ясена, рекомендації для лісового господарства.*