

Ash and ash dieback in the Czech Republic

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Introduction

At present, forestry often faces the misconducts of previous years. Based on principles of sustainable development forestry has got a far-reaching problem caused by much anthropogenic interference with the natural balance, which alters the structure and species composition of forests. The most important issues include decline and dieback of numerous tree species. Large-scale deforestation followed by forest management requiring production of profitable wood assortments shifted tree species out of its optimal ecological habitats. This approach keeps such forest stands under continuous abiotic stress enhancing their susceptibility to biotic agents. Together with many aspects of global changes trees, forests, plantations and woodlands are endangered by many new incidental factors, often acting in synergies and having significant influence in forest composition and stability of countryside. Extinction or decline of some woody plants has many other consequences, such as dramatic changes in ecosystems, changes in biodiversity due to disappearing of species, lack of traditional raw materials for industry and crafts with a considerable impact on traditions and culture.

In the Czech Republic ash is not a strategic tree species from the point of view of forest and wood production. However, it plays an important role in woodlands and its wood represents a very special material broadly processed by local joineries. Two native species, the common ash (*Fraxinus excelsior*) and narrow-leaved ash (*F. angustifolia*) form riparian vegetation and occupy significant positions in the floodplain forest ecosystem.

The main problems due to Ash dieback (ADB) can be expected in wetter sites with higher occurrence of ash, such as floodplain forests, riparian zones and spring sites (Havrdová et al., 2014). Furthermore, in riparian zones there is a risk of parallel attack of *H. fraxineus* and *Phytophthora alni* on ash and alder, respectively. Further problems can be expected in the ravine forest stands and protective forests on the slopes. The most serious losses occur to young individuals due to lethal course of infection. Also the permanent reduction of elm trees infected by Dutch elm disease produced by *Ophiostoma novo-ulmi* (Dvořák et al., 2007) reduces riparian tree composition. Alders, elms, and common ashes are the main woody plants in riparian forests with an important role stabilizing river banks, however, they are seriously threatened by these invasive pathogens.

Among forests ash is an important part of the cultural landscape in windbreaks, avenues and plays an important role in park's plantings. Because of this broad range of ash ecotops we can assume that *H. fraxineus* may cause significant problems. Although it mostly leads to death especially in young plantations (Rozsypálek, 2012), ash dieback spreads without distinction of stand's age. Natural forests, commercial plantations, forest nurseries, riparian vegetation, alleys, game reserves and urban plantings are attacked.

Importance of ash

Forestry, countryside, urban areas

By 2003 ash (*Fraxinus* spp.) was the sixth most common tree species in the Czech forests covering 1.7% of the forested area (National Forest Inventory, 2006). By 2014, the share of ash in the tree species composition of forests decreased to 1.4% (Ministry of Agriculture of the Czech Republic 2014), which can be attributed to the mortality caused by ash dieback. However, compared to 2004, when the disease was probably already present in the Czech Republic (Jankovský et Holdenrieder, 2009) ash proportion slightly increased. The stock of ash has been gradually increasing from 1990 until 2010 (FAO FRA, 2015) from 4.6 to 6.6 million m³, what classified it into the group of the top 10 forest tree species from the viewpoint of wood production. Before ADB ash was even mentioned as invasive species in some forest stands and it had to be eliminated from some protected areas.

Distribution of common ash in the Czech Republic ranges from lowlands to mountains. In lowlands it is key-stone tree in floodplain forests and on contrary also thermophilic oak stands on limestone slopes and steppes. In highlands ashes are common in alluvia of rivers, creeks and scree slopes, where can even reach mountain areas. The environmental importance of ash mainly consists of fulfilling the water management forest function, stabilizing the banks of streams and transpiration of large amount of water (Šimíček, 1999). In urban areas, such as alleys and parks, ash is the third most common tree followed by maples and limes. The species spectrum of ashes planted is wide but the common ash (*F. excelsior*) considerably prevails.

Tab. 1 Current composition of tree species in the Czech Republic (% of forest land area in the Czech Republic, 2004, 2009, 2014)

Woody plants	Natural composition	2004	2009	2014
Spruce	11.2	53.5	52.15	50.7
Pine	3.4	17.4	16.8	16.5
Fir	19.8	0.9	1.0	1.1
Beech	40.2	6.4	7.2	8.0
Oak	19.4	6.5	6.8	7.1
Larch	0	3.8	3.9	3.9
Birch	0.8	2.9	2.8	2.8
Alder	0.6	1.5	1.6	1.6
Ash	0.6	1.2	1.3	1.4
Maple	0.7	1.0	1.2	1.4
Hornbeam	1.6	1.2	1.2	1.3
Lime	0.8	1.0	1.1	1.1
Elm	0.3	0.0	0	0.0
Other	0.6	1.7	1.9	1.9

Source: Ministry of Agriculture of the Czech Republic, Forestry sector reports for years 2004, 2009, 2014.

Tab. 2 Actual composition (2015) of tree species in urban areas in the Czech Republic (%)

Maple	15.8	Pine	4.8
Lime	14.8	Oak	3.8
Ash	7.2	Poplar	3.5
Birch	6.8	Horse chestnut	2.5
<i>Prunus</i> varieties	6.6	Crane	2.3
Apple tree	6.0	Acacia	1.5
Spruce	4.8	Other	19.5

Wood industry

More than the half of the ash timber volume is used in the wood processing industry for production of furniture, parquet, veneer, musical instruments, tools and sporting goods, doors, stairs etc. Around 25% of the wood is used for paper production, 7% is used as fuelwood and the rest is used for other assortments of timber production. Use of ash timber in the Czech Republic has very long history, which persists until current times, when traditional materials are highly appreciated for special purposes. The ash wood is for example extraordinary resistant against vibrations because of its elasticity. Therefore, it has been used for skeletons of car bodyworks until early 1950's (Fig. 1 Aa), handles of tools, sport equipment (Fig. 1 Ac and Ad), short-range bows and arrows have been made from ash timber (for bows with longer range yew and cedar timber are more suitable). It was also used for the manufacture of skids in the sled, or sticks, baseball bats, boats and canoes. Many applications of ash timber are mostly forgotten and replaced by more advanced composites and technologies. The advantage of the unique appearance of ash wood has been taken by folk handicraft (Fig. 1 Ag) and furniture industry (Fig. 1 Ab) - ash timber can be ground and then rubbed into an almost perfectly smooth surface and production of resistant construction elements with exceptional design, such as doors (Fig. 1 Ae), stairs or floors. Other desirable products are ash veneer with a contrasting sapwood and heartwood. Particular properties of ash timber have also been used in the production of musical instruments; especially massive wood of ash, which is a common material in bass guitar bodies (Fig. 1 Af).

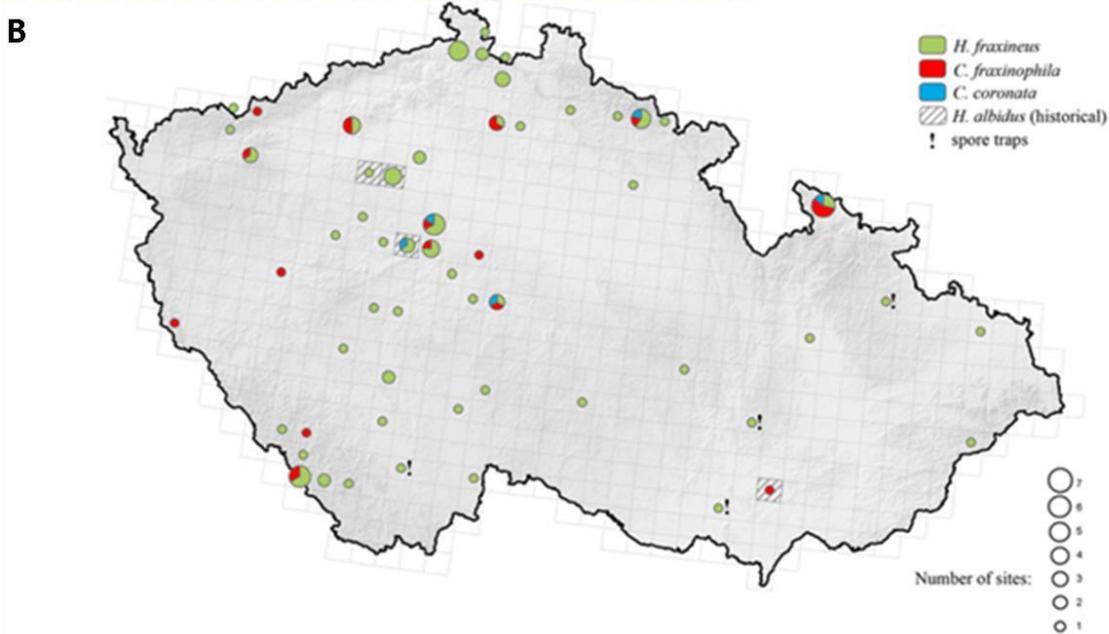
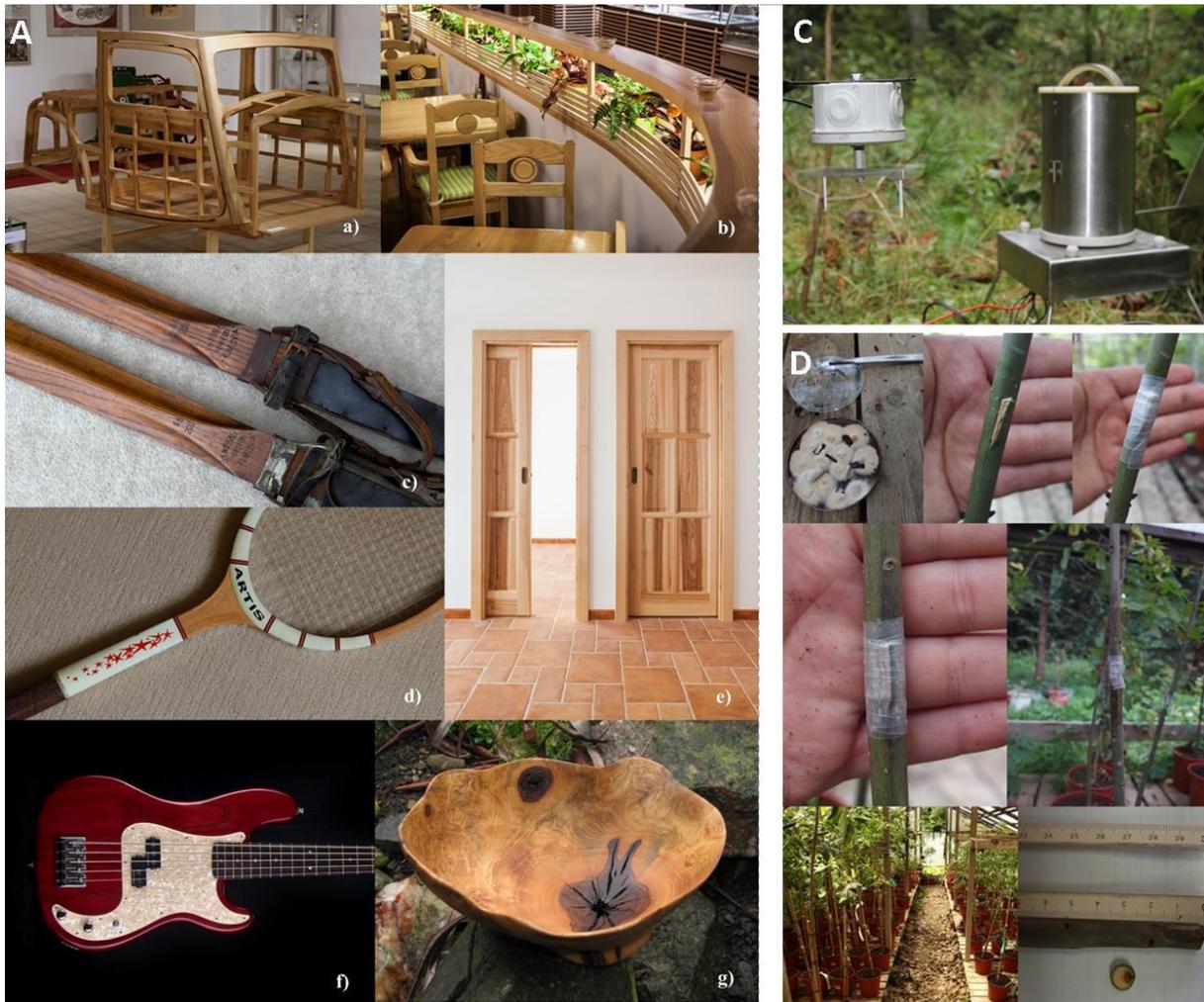


Fig. 1 **Aa)** Brand new replica (Truhlárství Jiří Hemzal, Vranov, Czechia) of bodywork sceleton for restored old-timer car Z-4 (Zbrojovka Brno, Czechoslovakia) produced in 1930's. Photo M. Dvořák; **Ab)** Furniture made of ash by Truhlárství Bočková (Žďárec, Czechia), Photo H. Bockova, <http://www.truhlarstvi-bocek.cz/>; **Ac)** Ski made of ash wood by Norgeski,Nossberger (Český Krumlov, Czechoslovakia) in 1930's. Photo Martin Tůma;

Ad) Tennis racket (Artis Štětí, Czechoslovakia) from 1970's. Photo M. Dvořák; **Ae**) Doors made of ash by Truhlářství Bočková (Žďárec, Czechia), Photo H. Bočková, <http://www.truhlarstvi-bocek.cz/>; **Af**) Bass guitar with ash body made by Prochazka custom guitars (Prague, Czechia), Photo K. Pazderka, <http://www.guitar-makers.com/>; **Ag**) Handicraft bowl made of ash (Brno, Czechia), Photo D. Palovčíková. **Fig. 1B**) Map of the *H. fraxineus* occurrence and *H. albidus* historical records detected by Koukol et al., 2016. **Fig. 1C**) Rotating arm (left) and volumetric (right) air samplers employed to sample the inoculum of ADB associated fungi. Photo M. Dvořák. **Fig. 1D**) Procedure of the *H. fraxineus* inoculation. Photo J. Rozsypálek.

Ash dieback and its spread (disease history)

In the Czech Republic symptoms of ash decline locally occur since the late 90s of the last century (there are some herbarium specimens from 2002 from Central Moravia) but since 2004 ash declining has remarkably increased. ADB appeared in Beskydy, Jeseníky, Giant mountains, the Bohemian forest, near Prague, eastern Bohemia, the Czech-Moravian highlands, Drahaný highlands, Hostýn in Chřiby (Jankovský et Holdenrieder, 2009). Symptoms of infection (such as drying of crowns, shoot dieback caused by the formation of necrotic spots under the bark) were recorded during the years 2004–2009 with varying intensity throughout the whole area of the Czech Republic (Jankovský et al. 2010).

The confirmation of *Hymenoscyphus fraxineus* in the Czech Republic was firstly reported from samples collected at Drahaný highland, arboretum Krtiny, from *Fraxinus excelsior* “Pendula” at the entrance to the arboretum, coordinates: 49°19'7"N/ 16°44'35"E (date of collection: 26th September 2007, sampled by O. Holdenrieder). The symptoms of ADB were already noted in this area in 2004 (Jankovský et Holdenrieder, 2009). Nowadays, *H. fraxineus* is commonly isolated throughout ash stands in the whole Czechia (Fig. 1B; Koukol et al., 2016).

Research regarding ash dieback

The ADB has been investigated thanks to various national research projects funded by the Czech Ministry of Education, Youth and Sports (RDIISCR, 2013), Ministry of Agriculture (RDIISCR, 2012) and by other European projects supported by COST (COST, 2011) and H2020 (EMPHASIS, 2015). Various topics have been developed at universities and research institutes. Below we shortly mention some of the investigated topics and their outputs.

Biology of the pathogen

Environmental factors affecting the epidemiology of *H. fraxineus* have been investigated by Havrdová et Černý (2013). Relationship of the ADB pathogen to the native saprobic species *H. albidus* and others was described by Koukol et al. (2016) and Dvořák et al. (2016), supported by RDIISCR (2012). Haňáčková et al. (2015) dealt with the population structure of *H. fraxineus*. A short work was conducted by Botella et al. (2016) to optimize a medium for isolation and cultivation of the ADB pathogen. Fructification, sporulation and symptoms of ADB in relation to phenology of ashes were studied on several stands in South Moravia within 2013–2015. Significant differences in the phenology, especially flushing time were found between severely and moderately infected trees (Rozsypálek, 2015a).

The spore dispersal pattern and influence of weather was investigated by Dvořák et al. (2016). The authors describe the ascospore dispersal pattern of both *H. fraxineus* and *H. albidus* in one locality in the Czech Republic. The most interesting highlight of this publication is the detected amount of inoculum apart of the expected spore dispersal season with the occurrence of sexual fruiting bodies (apothecia) i. e. April–October. Both species were continuously detected by volumetric and in few cases also by rotating arm air samplers (Fig. 1C). Samples from these instruments were processed by sensitive qPCR method. The possible reasons are discussed, supporting the suggestions of Gross et al. (2012) about the role of conidia in the reproductive process. Results of this work also support the relation between *H. fraxineus* and *H. albidus* inoculum co-occurrence with a clear prevalence of *H. fraxineus* during the whole sampling period, supporting the results of Koukol et al. (2016). Also the determination of inoculum occurrence by weather conditions is investigated. Authors have found significant influence of air humidity and leaf wetness on the inoculum amount with a certain time lag, suggesting the determination of fruiting bodies development and spore release by these factors (Dvořák et al., 2016).

The occurrence of putative, doubled-stranded (ds) viral RNA particles has been investigated in more than 100 Czech isolates (Čermáková, 2014). In total, three different dsRNA bands of *ca* 2.2, 2.5 and 4.5 kb were confirmed in 28.4% of the examined *H. fraxineus* samples (Čermáková, 2014). Statistical analyses have revealed no significant relation between the presence of dsRNA and the growth rate, colour or any other characteristic of the mycelium *H. fraxineus* (Čermáková, 2014). *H. fraxineus* mitovirus 1 (HfMV1) was recorded through high-throughput sequencing of dsRNA in one Czech isolate and confirmed to occur in all isolates presenting bands of *ca* 2.5 and/or 2.2 kb in size using direct specific retro-transcriptase (RT) PCR (Čermáková et al., in preparation). In cooperation with Dr. Daniel Rigling and Dr. Corine Schoebel (Swiss Federal Institute for Forest, Snow and Landscape Research, WSL) the population structure of *H. fraxineus* mitovirus 1 has been studied throughout Europe and Japan (Schoebel et al., in preparation). Furthermore, the presence of HfMV1 has been confirmed in *H. fraxineus* ascospores, which suggests ascospores as the main medium of HfMV1 transmission (Čermáková et Botella, 2015).

Development and impact of ash dieback

Distribution and impact of ADB in forest ecosystems together with the relation of stand conditions on infection intensity was monitored in 300 forest stands using GIS analyses. The ADB severity was assessed using the protocol developed by Rozsypálek (2015a) classifying trees into 10 health condition classes within which trees falling in class 1 and 2 (with lowest infection rate) are considered ADB-tolerant. Trees in class 9 (dead crowns), and 10 (collar and root infections) represent dead trees (Rozsypálek, 2012, 2015a; Prouza, 2015). Results show that severity of impact of ADB is decreasing with the altitude. The most significant differences in the intensity of ADB were noted in floodplain forests around Morava river. In the altitude higher than 760 m a.s.l. trees with lowest impact of ADB were registered (Fig. 2). Only mature pure ash stands were evaluated to avoid the influence of admixed species in early stage of the stand development. However, age of the stand seems to play an important role in the ADB severity of the stand. Young stands up to 50 years are significantly more affected by the ADB than mature stands (Fig. 3), where the trees are damaged with lower intensity (Prouza, 2015).

Symptoms of the ADB were followed weekly during 2013–2015 on experimental plots situated in lowland ash forests in the South-East of the Czech Republic. Heavy impact of the ADB has been recorded since 2008 throughout the whole area. Particular trees showed various dynamic of ADB symptoms development ranging from fast progress of the dieback to trees without any symptom's development. Probably due to extremely hot and dry season 2015 the development of the ADB symptoms was significantly retarded (Rozsypálek, 2015).

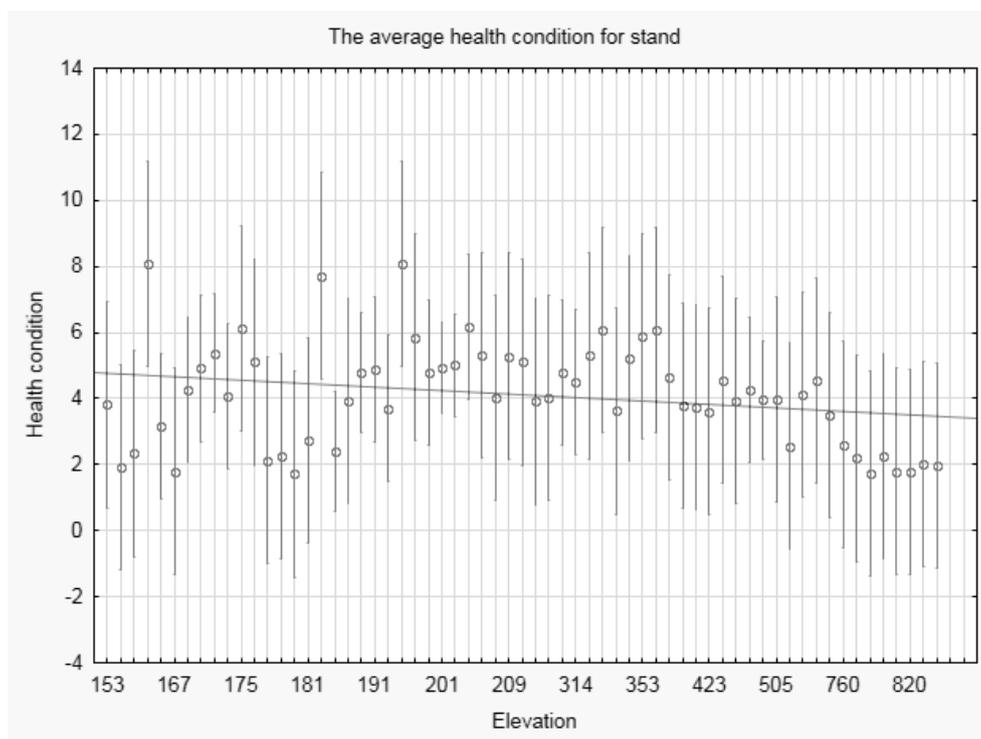


Fig. 2 Health of Ash stands according of altitude (Prouza, 2015).

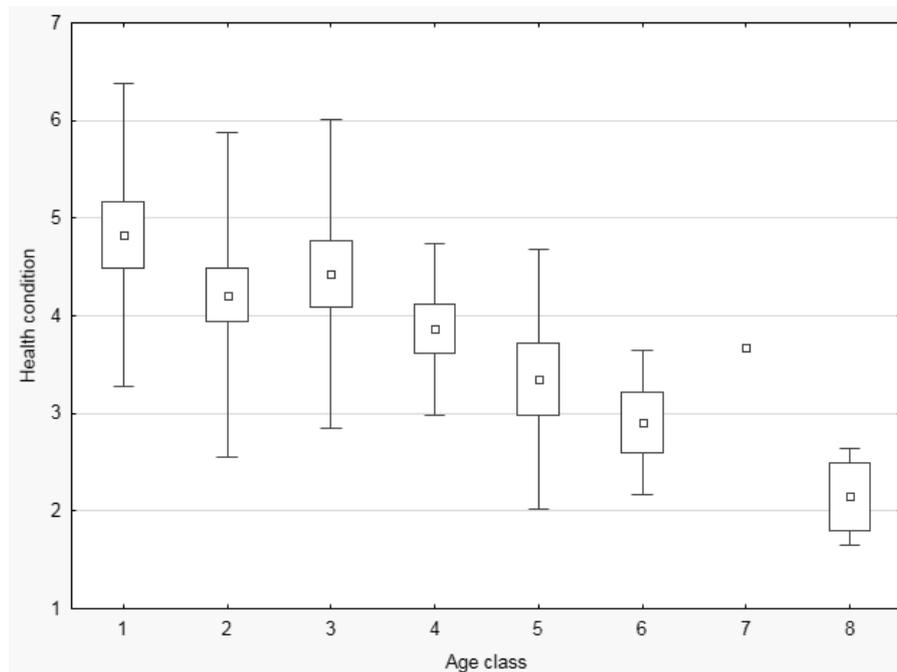


Fig. 3 Classification of health of ash stands according of age classes (1=0–20 years, 2=21–40 years...) (Prouza, 2015).

Genetics of ash populations and resistance

Susceptibility of ashes to *H. fraxineus* infection was studied by inoculation tests with 5 ash species and 4 cultivars. Inoculations were carried out with pure cultures and ascospore solutions of *H. Fraxineus* (Fig. 1D). Preliminary results confirm strong susceptibility of the species *F. excelsior*, especially cv. 'Pendula' and 'Altena', however cv. 'Nana' was evaluated as the least susceptible cultivar of this species. *F. americana* and *F. ornus* showed the highest tolerance among these experiments (Rozsypálek, 2015b).

Population structure and genetic diversity of ash in Czech Republic

The native Czech ash belongs to the Central European gene pool of species originated in the Alpine glacial refuge according to the phylogenetic studies developed by Heuertz et al. (2004a). The intraspecific variability of different phenotypic traits was studied in different ash provenances of Czech Republic. The experiment including 29 provenances replicated in 5 trial plots in different regions of the Czech Republic, and it was carried out during 1996–1999.

To evaluate the intraspecific variation of ash in relation to the ash dieback, the growth of provenances (heights and DBH of trees), their survival rates and health condition were compared. The health condition of the provenances (= dieback intensity) was assessed using the protocol developed by Rozsypálek (2015a).

Our preliminary results from three replicate plots of the experiment, which are located in eastern part of the Czech Republic (Tab. 3) indicate much better condition and growth of the narrow-leaved ash, which is native in the riverine sites along the Morava river. The two provenances of the species survive as much as twice better than the common ash provenances and their health condition is also significantly better.

Regarding genetic factors, provenance effects proved to be significant in all studied characteristics. They were the biggest in the survival rate and mean diameter of trees, followed by the mean height and health condition (= intensity of ash dieback). The survival rates of the best and poorest common ash provenances differed by 300%, their dieback intensity, phenotypic quality and mean diameter by 40%, and the mean height by 30%. These results are in accordance with Pliura et al. (2011), who reported 2 - to 4 - fold difference in the survival rate, and 50% in the health condition among populations in the geographically broadly based experiment covering Western, Central-European and East-European gene pools of the common ash.

Proportion of potentially resistant ash trees

We estimated proportions of hyposensitive trees of ash also at 5 trial plots of experimental plots established in 1996. The experiment is representative of the ash in the Czech Republic with regard to the number of provenances represented (29) and the fact that seeds of all provenances were collected personally by the founders in the source populations. In addition, there was not silvicultural intervention in 4 plots. Our preliminary results suggest no trend in the current survival rates (Tab. 3). On the other hand, proportion of nearly infection-free “hyposensitive” individuals (coinciding with the “expected final survival”) is obviously smaller in the plots located in the western part of the country (Bohemia) which may be with coinciding with climatic differences in the west-east direction.

Tab. 3 Current survival rate and expected final survival rate (based on the proportion of dieback-tolerant individuals) in the series of 5 provenance plots with 30 Czech ash provenances, age 20 years, established by the FGMRI Jílovište-Strnady.

Trial location	Habitat type	Parent substrate	Altitude	Survival rate - 19 years	Expected final survival*
SE Moravia - Tvrdonice	riparian mixed forest	riverine sediments	155	21,20%	7,20%
Central Moravia - Kroměříž	riparian mixed forest	riverine sediments	190	25,20%	5,50%
NE Bohemia - Deštná	Alder-Ash alluvial forest	sandstone sediments	250	14,80%	1%
Central Bohemia - Koněprusy	Tilio-Acerion slope forest	limestone	350	23,15%	1,80%
East Moravia - Vápenky	Tilio-Acerion slope forest	calcarous flysch	490	21,90%	4%

* Ratio of hyposensitive trees (Class 1 and 2 in the scale of 10) to all originally planted trees in a trial

Management options to mitigate the impact of ash

Silvicultural management options

In young ash, the dieback progresses considerably faster than in adult trees (Rozsypálek, 2015a) because of the size of the host tree but probably also due to higher humidity and higher concentration of spores closer to the ground surface and in understory of forest stands.

However, young stands of the thicket and pole-stage appear to be more threatened by the changed attitude of forest managers towards the species. The indirect effects include direct elimination of the whole groups of declining ash trees including infection-tolerant individuals within them and reduced interest in the silvicultural support to the species by thinning of the species mixtures. Drop in the number of healthy ash seedlings available in the forest nurseries contributes to the overall trend of avoiding ash in the reforestations.

Phenological investigation focused on ashes with different degree of damage by ADB showed clear differences. Severely damaged trees showed a time lag of the spring phenological phases up to one month compared to the healthy trees (Rozsypálek, 2015a). This apparent influence of the pathogen on the seasonal development of the particular trees can indicate targeted silvicultural measures in the spring time. Thus trees with delayed flushing can be removed before further development of the ADB symptoms.

In the both riparian and highland ash sites, potential effects of thinning variants have been tested under the following premises:

- timing (winter and summer thinning);
- thinning method (conventional sanitary thinning aimed to elimination of mean and inferior individuals and positive thinning to support the most vital ash trees).

The hypothesis is that thinning of susceptible individuals in a larger area in winter may have an indirect positive effect thanks to the lower quantity of infected leaves and putatively reduced ascospore discharge in the next vegetation season(s). The effects of thinning will be estimated evaluating the growth, infection intensity and overall health in the thinning variants and the non-intervened plots.

Possible control measures

Due to the rapid spread and high infection pressure of *H. fraxineus* the possibilities of ADB control are limited. In the forest nurseries it is possible to use chemical treatment to protect planted material. But these measures are ineffective, because the plants are protected only when remaining in the nursery. After planting those seedlings into the forest they are heavily damaged and the infection pressure increases in the surrounding vegetation. Chemical control is useless in forest stands. Fungicides used in the large scale can lead to disbalances of macro and micronutrients in the soil and pollution of water sources (Forestry commission, 2005), regardless to the economic impact. The use of fungicides is acceptable only for individual control of valuable trees in countryside and in urban areas.

It seems that the only possibility to preserve ash trees in forests is searching and breeding individuals with increased genetic resistance to *H. fraxineus*. Despite the enormous infection pressure in almost all ash stands there is always a small percentage of minimally affected individuals (Stener, 2012).

In forest ecosystem is appropriate to support those ashes with minimal symptoms and its genetic potential for natural regeneration in stands. Breeding programs with resistant trees are recommended in alleys, river banks, urban areas and artificial plantations. Unfortunately local foresters prefer simple solutions and ash silviculture is dramatically restricted now. Artificial regeneration of ashes is not recommended nowadays because of the high rates of mortality (100%).

So far, biological control does not seem to be a plausible solution to improve the situation of ashes in the Czech Republic. Preliminary investigations carried out to assess a possible effect of fungal viruses on the morphology and growth rate of their fungal hosts did not show statistical significance (Čermáková, 2014). Nevertheless, further experiments should be performed to truly understand the role of mycoviruses within *H. fraxineus*.

Alternative tree species

Severe dieback of trees species, such as alder, elms and ashes has a serious effect on tree diversity. In the softwood floodplain forests genera willows (*Salix* spp.) and poplars (*Populus* spp.) are widely spread but they may suffer from other pathogenic agents as *Dothichiza populea*, *Melampsora* spp., *Xanthomonas populi*, *Agrobacterium* spp. etc. Also native pedunculate oak (*Quercus robur* L.), which remains as the main tree species in the hardwood floodplain forest, shows a significant decline in many localities likely because of climatic and hydrologic reasons. Wild cherry (*Prunus avium* L.) is suitable additional tree species in these stands as well.

Due to decline and dieback of native woody plants in floodplain forest, the importance of other woody plants, including not native species, is increasing. Ash is often replaced by black walnut (*Juglans nigra* L.), but in some habitats there is major risk of introduction of thousand canker diseases caused by *Geosmithia morbida* and transmitted by the walnut twig beetle *Pityophthorus juglandis*. Honey locust (*Gleditsia triacanthos* L.) is used occasionally. The floodplain forest niche vacated by the retreat of ash trees is naturally overgrown with boxelder (*Negundo aceroides* Moe.) and hedge maple (*Acer campestre* L.). However use of non-native trees species is strictly limited by forest and environmental legislation.

The lack of restriction in the use of non-indigenous species in the urban environment is enhancing the ash replacement. To preserve ash in urban environment it is recommended to plant more resistant species and cultivars of ash, such as manna ash (*Fraxinus ornus* L.) or cultivars *F. excelsior* 'Nana', which seems to be more resistant. The use of some cultivars is limited by the absence of suitable resistant rootstock for grafting. Some non-native ashes like *F. pennsylvanica* should be also considered (Rozsypálek 2015b).

Conclusions

Ashes in the Czech Republic are until certain point replaceable either from the point of view of wood production, either from the ecological view. To forget ash means to avoid a natural resource, which is historically connected

with the culture of the Czech Republic. This traditional material would be missed in many branches of production. Companies and craftsman working with ash timber were never focused on quantity, but on high quality. Design of ash products is mostly highly personalized. Therefore we cannot give up the fight against ADB, support silviculture on the bases of natural regeneration in wide scale and keeping the high genetic diversity.

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