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European Ash (*Fraxinus excelsior* L.) Dieback: Disintegrating forest in the mountain protected areas, Czech Republic

Absterben der Gewöhnlichen Esche (*Fraxinus excelsior* L.): zerfallende Wälder in Bergschutzgebieten, Tschechische Republik

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Key words: Health condition, *Hymenoscyphus fraxineus*, dynamics, Giant Mountains, defoliation, Central Europe

Schlagworte: Gesundheitszustand, *Hymenoscyphus fraxineus*, Dynamik, Riesengebirge, Entlaubung, Mitteleuropa

Abstract

European ash (*Fraxinus excelsior*) is an important tree species in most temperate forests in Europe. Its future is threatened however, especially by an invasive fungus, *Hymenoscyphus fraxineus* (*Hymenoscyphus pseudoalbidus*, *Chalara fraxinea*). The current study is focused on the health of ash in the Krkonoše Mountains National Park, Czech Republic. On permanent research plots containing mixtures of ash and other species, the stands' health condition was evaluated annually in 2009–2015, by using radial increment and foliage of 350 trees as indicators, with emphasis on the cenotic arrangement and morphological type of the crown. The results show mean annual decrease in foliation of 1.6–2.7% for live trees and 2.4–4.6% in all live and dead trees, with an

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overall mortality of 16.3% of trees. During the latter half of the study, the defoliation dynamics and dieback accelerated, especially at lower altitude and waterlogged sites. In 2012–2015, the mean annual decrease in foliation reached 3.9% in live trees and 6.3% in all trees. The mean radial increment was significantly lower 2 years after the purported fungi attack and the mean foliage density was significantly lower 6 years after the attack compared to healthy trees. Based on the evaluation of the results and the literature, a set of practical guidelines was established for management strategies and conservation of threatened European ash in similar stand conditions.

Zusammenfassung

Die Gewöhnliche Esche (*Fraxinus excelsior*) ist eine bedeutende Baumart in meisten Wäldern der Gemäßigten Zone Europas. Ihre Zunkunft ist jedoch bedroht, und zwar wegen des invasiven Pilzes *Hymenoscyphus fraxineus* (*Hymenoscyphus pseudoalbidus*, *Chalara fraxinea*). Das ist ein Grund, weshalb sich diese aktuelle Studie auf einen Gesundheitszustand der Gewöhnlichen Eiche fokussiert hat. Auf gemischten dauerhaften Eschenforschungsgebieten wurde eine Dynamik des Gesundheitszustandes in Jahren 2009-2015 jährlich ausgewertet, wobei ein radikaler Zuwachs und eine radikale Belaubung von einzelnen 350 Bäumen als ein Kriterium betrachtet wurde, und zwar in Hinsicht auf eine coenotische Stellung und einen morphologischen Typ der Krone. Ergebnisse zeigen eine durchschnittliche jährliche Entlaubung 1.6-2.7% im Falle der lebendigen Bäume und 2.4-4.6% bei allen lebendigen und toten Bäumen zusammen. Die Gesamtmortalität der Bäume erreicht bis zu 16.3 %. In der zweiten Hälfte des Zeitraums wurden Dynamik und Entlaubung schneller, insbesondere dann in niedereren Höhen über dem Meeresspiegel auf durchfeuchten Lokalitäten. In den Jahren 2012-2015 hat die durchschnittliche jährliche Entlaubung bei lebendigen Bäumen 3.9% und bei allen Bäumen 6.3% erreicht. Nach einer Chronologie der Jahresringe wurde ein bedeutend um 2 Jahre niederer radikaler Zuwachs festgestellt. Die Ursache liegt vermutlich im Befall von Pilzen. 6 Jahre nach diesem Befall wurde die durchschnittliche Belaubung bedeutend niederer. Aufgrund der Auswertung der Ergebnisse und aufgrund der literarischen Quellen war eine Gesamtheit der praktischen Weisungen für Strategien des Managements und für einen Schutz der gefährdeten Gewöhnlichen Esche festgesetzt.

1. Introduction and problem analysis

European ash (*Fraxinus excelsior* L.) plays an important role in both primary and secondary succession, resulting in its presence across vast areas of primary and secondary forest of all age categories (Dobrowolska et al. 2011). In mixed deciduous forests, it is often accompanied by European beech (*Fagus sylvatica L.*), Durmast oak (*Quercus petraea* /Matt./ Liebl.), English oak (*Quercus robur L.*), sycamore maple (*Acer pseudoplatanus L.*), black alder (*Alnus glutinosa* /L./ Gaertn.) or speckled alder (*Alnus incana* /L./ Moench). Biological demands and ecological characteristics of European ash have been investigated in various studies (e.g. Wardle 1961; Thill 1970; Wagner

1997; Emborg 1998; Kerr and Cahalan 2004; Götmark et al. 2005; Střeštík and Šamonil 2006).

In Europe, European ash has been considered an expansive tree species in the last 100 years for its ability to quickly cover open areas (Ellenberg 1996; Emborg et al. 2000). Earliest records of the European ash expansion come from England dating back to the turn of the 19th and 20th century (Adamson 1921; Watt 1924). The phenomenon of European ash expansion might be related to agricultural abandonment in the countryside (Diekmann 1999; Marigo et al. 2000). According to Hofmeister et al. (2004) it is also related to increasing nitrogen deposition. The expansion of European ash is aided by its considerable competitiveness at the juvenile stage and, particularly, by its fast proliferation into canopy gaps (Le Goff and Ottorini 1996; Hofmeister et al. 2004). Young European ash trees also profit from their drought stress tolerance (Ryšavý and Roloff 1994; Horn 2002).

In the past, the genus *Fraxinus* was a problem-free tree species, not susceptible to diseases, fungi, or insect pests (Skovsgaard et al. 2010). During the past twenty years, though, populations of European ash have been dwindling in many locations across Europe and its expansion has slowed (Bakys et al. 2009b). At present, European ash faces many threats (Dobrowolska et al. 2011). Among insect pests, a particular threat is the emerald ash borer, *Agrilus planipennis*, an East Asian wood-boring beetle which can kill more than 85% of healthy ash trees in a forest stand within 3 to 5 years (Poland and McCullough 2006). In North America, the beetle has killed tens of millions of ash trees (Siegert et al. 2007). Damage and mortality due to the emerald ash borer greatly alters canopy density and lead to profound stand increment changes (Kashian and Witter 2011). The emerald ash borer has not been detected in the Czech Republic so far and at this time does not pose an immediate threat (CITIA 2014) in comparison to the fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, Hosoya, comb. nov. (Baral et al. 2014; synonym: *Hymenoscyphus pseudoalbidus* Queloz et al.; basionym: Chalara fraxinea T. Kowalski). This fungus is responsible for extensive dieback of ash trees and a dramatic decline in their number (Orlikowsky et al. 2011). Ash tree dieback caused by *H. fraxineus* was first recorded in Poland in the 1990s (Kowalski 2006) and the fungus has quickly spread into Eastern, Central and Northern Europe (Skovsgaard et al. 2010). The disease drastically affects ash trees of all age categories and mortality is very high; therefore not only ash trees but also dependent species are threatened (Pautasso et al. 2013a).

Withering of an ash tree infested by *H. fraxineus* is manifested by wilted leaves, dark brown leaf-stalk necrosis, premature defoliation of green leaves, dieback of young annual shoots, and under-bark necrosis (Kowalski 2001; Przybyl 2002; Barklund 2006; Thomsen and Skovsgaard 2006; Thomsen et al. 2007). At first, the symptoms appear mainly in the tree crown and often result in dieback. Later, the outer bark on the trunk turns reddish (Kowalski and Lukomska 2005). Necrosis has been observed in trees of average or below-average size in the stand, in other words, those of lower competitive ability (Skovsgaard et al. 2010). Wilting and dying of ash trees also depends on the site and forest stand conditions and on foresters' management practices (Kowalski 2006; Bakys et al. 2009b; Dobrowolska et al. 2011). Confounding the analysis, damaged trees have been found to be infected by other fungi as well (especially Armillaria lutea Gillet) and bark beetles (e.g., *Hylesinus fraxini* Panzer or *H. varius Fabricius*) (Heydeck et al. 2005; Kowalski and Lukomska 2005; Bakys et al. 2009b).

The extent of the impact of *H. fraxineus* on European ash can be compared with that of *Cryphonectria parasitica* on American chestnut; this fungus killed an estimated 3.5 billion chestnut trees, especially in forests in the eastern United States (Kashian and Witter 2011). In North America and Europe, a similar destructive process was observed when elm trees were infected by Dutch elm disease (*Ophiostoma novo-ulmi*) (Solla et al. 2005).

The purpose of this study was to evaluate the health condition of forest stands with a large European ash component at various sites and with various proportions of ash within stands resulting from secondary succession on former farmland along an elevational gradient of the Krkonoše Mountains in 2009–2015. Our objectives were to 1) identify the long-term trends in health status (foliation) and productivity (radial growth) of European ash stands affected by *H. fraxineus*, 2) identify interactions among site characteristics, health status and stand indicators to the range of damage and 3) propose possible measures to reduce, restore from or prevent damage caused by the fungal pathogen in similar site conditions.

2. Material and Methods

2.1. Study area

The research was carried out on 4 permanent research plots (PRPs) 50 × 50 m (0.25 ha) in the Krkonoše National Park, established in 1963, in the Czech Republic. Elevation gradient of PRP ranges from 540 to 860 m a.s.l. in the eastern part of the park. The parent rock of this park is formed mainly by granite, mica schist and phyllite. At the lowest altitudes predominant soil types are Cambisols, above 1,000 m Podzols and in the proximity of numerous water springs the Gleysols. Average precipitation varies with respect to altitude from 650 mm to 1,260 mm per year and mean annual temperature decreases with altitude from 7.6 °C to 2.6 °C. The location of the PRPs is shown in Figure 1; information about the PRPs is summarized in Table 1.

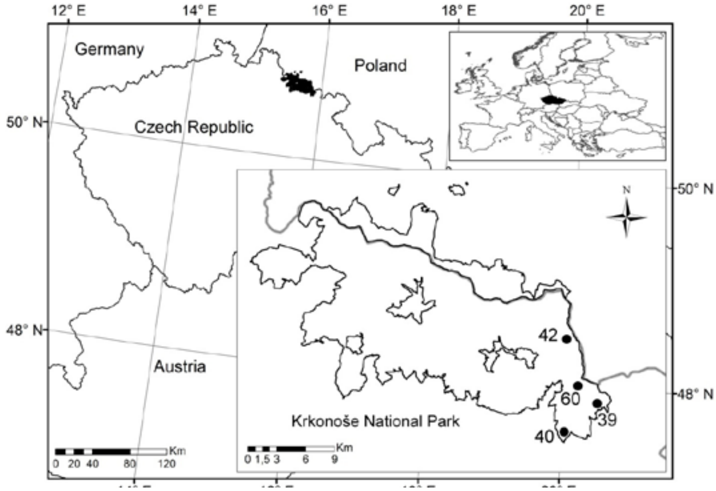

Figure 1: Location of forest stands with European ash on permanent research plots in the Krkonoše Mts. (GPS: PRP 39 – 50º39'03"N, 15º 53'19"E, PRP 40 – 50º37'08"N, 15º51'01"E, PRP 42 – 50º42'30"N, 15º50'01"E, PRP 60 – 50º39'51" N, 15º51'07"E)

Table 1: Overview of basic site and stand characteristics of permanent research plots

PRP	Altitude (m)	Veg. season	T (ºC)	Precip. (mm)	Soil type	Aspect	Slope (º)	Forest type[2]	Age (y)	Volume ($m^3.ha^{-1}$)	Species (%)[1]
39	650	124	12.8	640	Histic Gleysols	NE	14	5V	78	496	AG 33, FE 26, AP 18, FG 16, UG 5
40	540	130	13.9	610	Modal Cambisols	SW	4	5D	80	601	FE 59, AP 32, AA 5, FS 2, SA 2
42	850	115	10.8	660	Gleyic Cambisols	SE	24	6D	59	396	FE 48, PA 30, AG 17, AP 3
60	860	111	10.1	650	Gleyic Cambisols	NE	16	6D	62	371	FE 54, AP 41, AG 3

Explanatory notes: [1]species composition > 1 %: AA – Abies alba, AG – Alnus glutinosa, AP – Acer pseudoplatanus, FE – Fraxinus excelsior, FS – Fagus sylvatica, PA – Picea abies, SA – Sorbus aucuparia, UG – Ulmus glabra;
[2]forest site type: 5V – moist fir-beech zone, 5D – enriched fir-beech zone, 6D – enriched spruce-beech zone. In view of potential vegetation, the 5V group of forest habitat types is a brook and spring alder zone of the suballiance Alnenion glutinoso-incanae Oberdorfer 1953 and the 5D and 6D GFHT represent vegetation associations of Pruno padi-Fraxinetum excelsioris Oberdorfer 1953 and Mercuriali perennis-Fraxinetum excelsioris (Klika 1942) Husová in Moravec et al. 1982.

2.2. Data collection

The health condition of the European ash mixed stands on the 4 PRPs in 2009–2015 in the Krkonoše National Park was evaluated annually in August based on description of the foliage of individual trees (n=350) and categorization of the degrees of defoliation (Table 2). The mean foliage of the stand is expressed as an arithmetic mean of foliage values of all trees on a PRP and of live trees (cf. Vacek et al. 2013). Defoliation (complementary to foliage up to 100%) with an emphasis on the coenotic position and diameter structure was assessed with the accuracy of 5% and recorded using six degrees of defoliation, corresponding to the degrees of tree damage. These methods are almost identical to those used in the ICP-Forests and ICP-Focus international project (Lorenz 1995).

Foliation of each tree species was evaluated individually, in relation to coenotic position of the tree (trees: 1 – dominant, 2a – co-dominant principal, with a well-developed crown, 2b – co-dominant secondary, with a crowded crown, 3 – sub-dominant, 4 –suppressed, shade-grown viable, 5 – dying and dead; Konšel 1931), tree crown quality (1 – oversized /above average/, evenly developed, symmetrical /with dense foliage/, 2 – average-sized, unevenly developed with sufficiently dense foliage, 3 – average-sized, unevenly developed or with insufficiently dense foliage, 4 – small, with serious malformations and thin foliage, 5 – very small, poorly developed with thin foliage) and classification of canopy exposure (insolation degrees; crown: 1 – exposed from all sides, in no contact with neighbouring trees, 2 – crown touching neighbouring trees at one side, 3 – crowded from two sides, 4 – crowded from three sides, 5 – crowded from all sides; Assmann 1961).

Soil samples were collected on individual PRPs in September 2014. Intact soil samples were taken by using Kopecky cylinders in different soil horizons for analysis of physical properties. Momentary soil moisture content and moisture content (Kutílek and Nielsen 1994) were measured in each sample.

Table 2: Defoliation degrees and their characteristics (Vacek et al. 2013)

Degree of defoliation	*Foliation (%)*	*Defoliation (%)*	*Tree characteristics*
0	91–100	0–9	Healthy
1	71–90	10–29	Slightly damaged
2	51–70	30–49	Medium damaged
3	31–50	50–69	Seriously damaged
4	1–30	70–99	Dying
5	0	100	Dead

Comparative radial growth analyses were conducted using tree cores taken at a height of 1.3 m (DBH) using a Pressler auger from 15 trees without dieback symptoms (defoliation < 15 %) and 15 trees with symptoms (defoliation > 60 %) for ash trees of average height and above-average height in each PRP (a total of 120 samples). The annual ring width was measured to an accuracy of 0.01 mm using an Olympus binocular magnifying glass on a LINTAB measurement table and registered using the TsapWin software (www.rinntech.com).

Five shoot cuttings from trees infected with *H. fraxineus* were taken from each PRP in May 2015 in order to isolate the fungus. The isolation of fungi from shoots (June 2015) was performed from discolored necrotized wood following the methods of Kowalski (2006). A total of twenty fungus cultures were isolated on culture medium in Petri dishes. For retaining isolation, wort agar-based medium (MEA) with the addition of streptomycine antibiotics after sterilization by ethanol and sodium hypochlorite was used. The subsequent culture of isolates was carried out in darkness for one month at 20-22°C. Afterwards, the cultures were transferred to wort agar (MEA) without streptomycine. An Olympus BX41 microscope with an Olympus Camedia C-5060WZ camera was used for documentation of cultures.

2.3. Data processing

To aid further calculations, the degrees of foliation were transformed to proportional values (mean values for each degree of defoliation). The health condition of the European ash trees was evaluated based on the development of the arithmetic mean foliage of all live and all trees (dead + living) on the PRP, standard deviation of defoliation and the development of the number of dead trees (completely defoliated trees). For an overall evaluation of stand health condition, total means of foliage of all trees, including the completely defoliated trees, were also calculated. The current study evaluates the health condition of European ash only.

To calculate tree radial growth, the annual ring increment series were cross-dated individually (eliminating errors caused by missing annual rings) by using statistical tests in the PAST4 application software (Knibbe 2007) and then subjected to visual inspection according to Yamaguchi (1991). If a missing annual ring was found, a ring 0.01 mm wide was inserted in its place. The individual curves were then detrended and an average annual ring series was created using ARSTAN software. The 60-year spline was applied (Grissino-Mayer et al. 1992).

Statistical analyses were processed in the Statistica 12 software (StatSoft, Tulsa). Data were log transformed to a normal distribution (tested by Shapiro-Wilk test). The differences in mean annual radial growth and mean annual foliage of trees among plots were tested by one-way analysis of variance (ANOVA). Significantly different results were then tested by the post-hoc HSD Tukey test.

An unconstrained principal component analysis (PCA) in the CANOCO 4.5 program (Ter Braak and Šmilauer 2002) was used to analyse relationships among plots attributes, stand parameters, climate data and health condition of *Fraxinus* and similarity of the 4 research plots over time. Data were centred and standardized during the analysis. The results of the PCA analysis were visualized in the form of an ordination diagram constructed by the CanoDraw program (Ter Braak and Šmilauer 2002).

3. Results

3.1. Foliage as an indicator of health condition

The development of mean foliage of all trees and mean foliage of live trees as evidenced by the share of defoliation degrees of European ash on PRP 39, 40, 42 and 60 from 2009 through 2015 is summarized with respect to the actual stand composition on each PRP (Fig. 2).

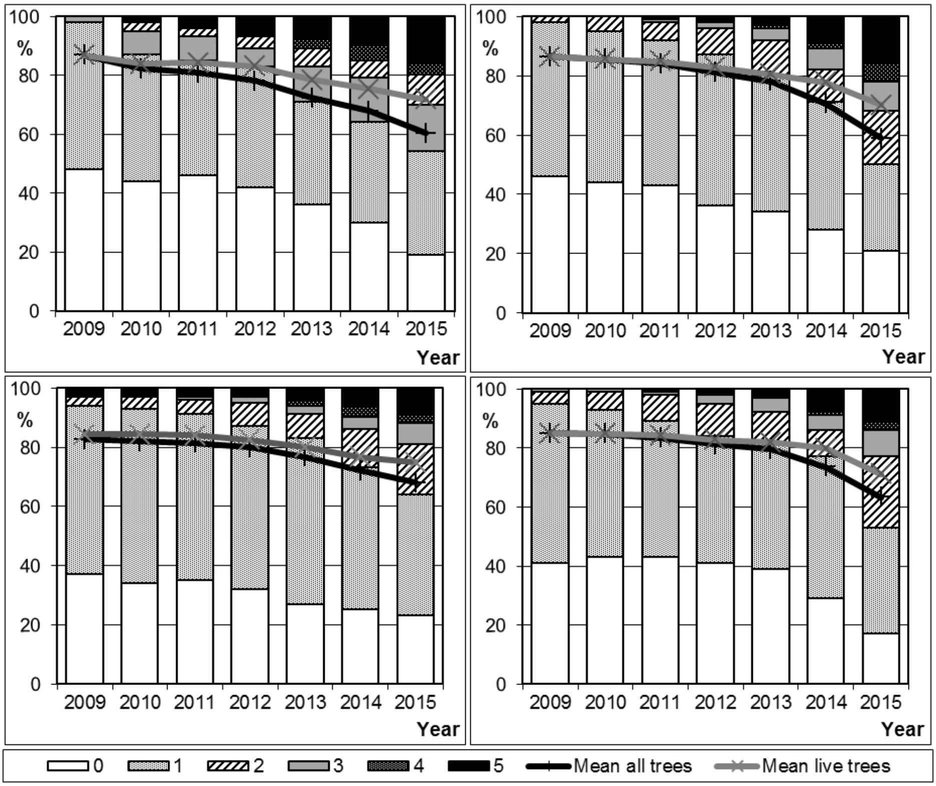

Figure 2: Trends in the mean annual foliage of all (black line) and live ash trees (grey line) and degrees of defoliation (1-5) in the mixed forest stand on permanent research plots

The development of trees foliage on PRP 39 with the high soil moisture suggests that in 2009–2011 the health of live ash trees (Fig. 2) was relatively stable. The mean annual decrease in foliation of live trees was 1.2% and 2.9% for all trees (annual mortality 1.7 %). Since 2010, though, a distinct defoliation of co-dominant trees with crowded crown has been observed. In 2012–2014, the dieback increased considerably and significant defoliation also affected dominant trees with an unevenly developed crown crowded from several sides. The annual decrease in foliation in 2012–2015 was 2.5% and 5.2 % in live trees and all trees, respectively (mortality 2.7%). By 2015, defoliation began to affect dominant trees with evenly developed crowns as well. Before 2014, distinct defoliation of trees on PRP was only found on individual trees, but in 2014, it was grouped in bigger patches. In 2015 total mortality of ash trees reached 15.8% during the observed period.

On lowest elevation site PRP 40, it appeared that the ash trees (Fig. 2) were fairly healthy in 2009–2011 with only moderate defoliation of sub-dominant trees with a small crown. The mean annual decrease in foliation in that period reached 0.8% in live trees and 1.2% in all trees (annual mortality 0.4%). In 2012, the defoliation accelerated slightly; co-dominant trees with a small and crowded crown began to die. In 2013, advanced defoliation began to occur in dominant trees with unevenly developed crowns. In 2014 dominant trees with an unevenly developed crown began to die. In 2012–2015, the annual decrease in foliation reached 3.7% in live trees and 6.3% in all trees (mortality 2.6%). In 2014 the advanced defoliation expanded in groups. By 2015, the total mortality of the ash trees on PRP 40 reached 16.3%.

The European ash trees were fairly healthy in 2009–2012 (Fig. 2) with only moderate

Figure 3: Ash tree highly affected by the fungus ***H. fraxineus*** with considerable dieback of branches in the crown reacts by developing a large proportion of epicormic shoots even after major local necrotic damage to the bark

defoliation of sub-dominant trees on PRP 42. The mean annual decrease in foliation in that period reached 0.5 % in living trees and 0.9 % in all trees (annual mortality 0.4%). In 2012, the defoliation trend accelerated slightly; advanced defoliation occurred only in sub-dominant trees with crowded crown. Trees began to die due to *H. fraxineus* attack. At that time, only trees with strongly suppressed growth, without symptoms of the fungus, were dying (Fig. 3). In 2015, advanced defoliation was occurring in co-dominant trees but also began in dominant trees. In 2012–2015, the annual decrease in foliation reached 2.5% in live trees and 3.9 % in all trees (mortality 1.4%). The advanced defoliation was still only random. During the observed period mortality of ash on PRP 42 was the lowest – 9.2%.

On PRP 60 with the highest altitude the ash trees were also fairly healthy in 2009–2012 with only moderate defoliation of sub-dominant trees with a crowded crown (Fig. 2). The annual decrease in foliation during this period reached 0.7% in live trees and 1.3% in all trees (annual mortality 0.6%). In 2013, defoliation accelerated only in co-dominant trees, mostly with a suppressed crown. In 2014 and 2015, advanced defoliation also occurred in dominant trees with unevenly developed crowns crowded at the sides. In 2012–2015, the mean annual decrease in foliation reached 3.9 % in live trees and 6.0% in all trees (mortality 2.1%). The advanced defoliation on PRP was still random. In 2015 mortality of the stand reached 10.8%.

Comparing all plots, the mean foliage of all individuals were not significantly different in 2009 ($F_{(3, 346)} = 1.8$, $P > 0.05$). The highest mean foliage was found on PRP 40 (86.3 ± 1.6 SE). The lowest mean foliage was found on PRP 42 (84.4 ± 1.2 SE). Comparing all plots in 2015, there was no significant difference between the mean foliage ($F_{(3, 346)} = 2.1$, $P > 0.05$), but separately the mean foliage was significantly higher on PRP 42 (68.1 ± 3.3 SE) than on PRP 40 (58.9 ± 3.2 SE, $P < 0.05$). The mean foliage of all trees was significantly higher in 2009 than in 2015 ($F_{(1, 698)} = 88.4$, $P < 0.001$), and the mean foliage of all live trees was significantly higher in 2009 than in 2015 ($F_{(1, 654)} = 46.5$, $P < 0.001$). Between 2009 and 2015, the smallest change in mean foliage was observed on PRP 42 (14.6%), and the largest on RPR 39 (26.6%). During these 6 years, the highest mortality of ash trees was also observed on sites at lower elevation (< 650 m), more flat terrain (slope < 14°), larger total stand volume (> 496 $m^3.ha^{-1}$) and higher soil moisture (mortality on PRP 40 16.3%, PRP 39 15.8%) compared to high-elevation plots (> 850 m), greater slopes (> 16°), lower productivity (< 396 $m^3.ha^{-1}$) and drier sites (PRP 42 9.2%, PRP 60 10.8%).

3.2. Dynamics of radial growth

The mean annual increments of healthy ash trees and trees infected by *H. fraxineus* after removing the age trend of all trees are displayed in Fig. 4. The comparison of the average annual ring curves of ash across the four PRPs indicates a high rate of agreement among them (t-tests > 4.6). These results allowed us to compile a local standard chronology for the spruce stands in the ash mixed stands of the Krkonoše Mts.

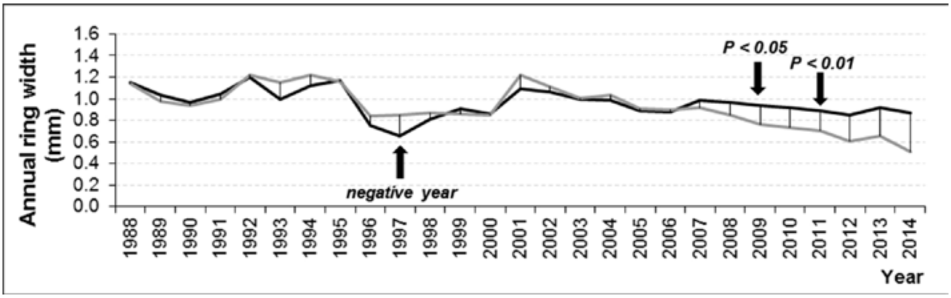

Figure 4: Mean annual ring increment in the entire study area of ash trees without dieback symptoms (black line) and trees with symptoms of ***H. fraxineus*** (grey line) after removing the age trend in Arstan software; black arrows indicate significant negative pointer year and significant differences between ring increments of two study groups

The regional annual ring chronology for healthy trees and infected trees by *H. fraxineus* indicates a relatively balanced radial increment until 2006 (the year of supposed attack by fungi), interrupted by several small fluctuations in 1993 and 1997 and then again in 2001. The period of decreased radial increment of infected trees started in the 2007. According to ring analyses, 2 years after the attack (2008) by fungi, the

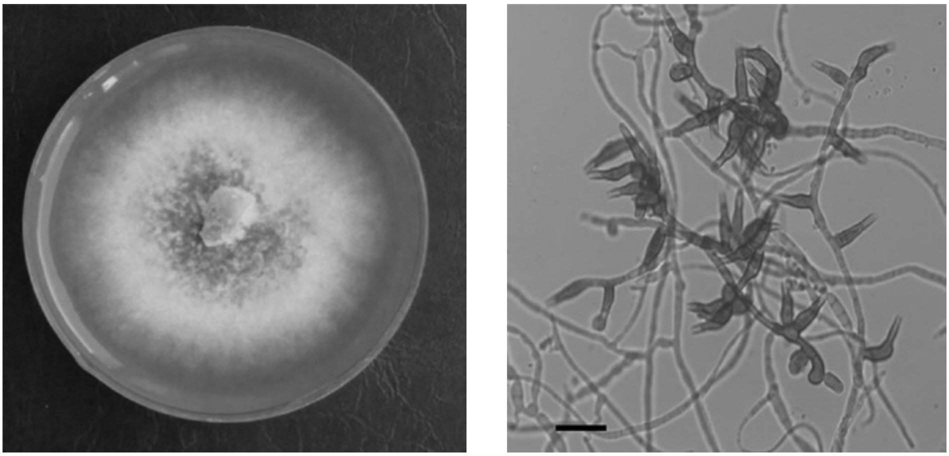

Figure 5: Colony of ***Hymenoscyphus fraxineus*** (left); Phialides and conidia, bar 20 µm (right)

mean annual radial increment of infected trees was significantly lower than that of the vital trees ($F_{(1, 109)} = 4.1$, $P < 0.05$). For example, an average annual ring width of healthy trees without damage by fungi at this site is about 1.49 cm (± 0.15 S.E) for stand age 64 (2014), but under heavy attack by *H. fraxineus* it is only 0.52 cm (± 0.09 S.E.) wide (i.e., 35% of the former width).

On the other site used for comparing foliage dynamics, the mean foliage of all trees was significantly higher in 2009 than in 2012 ($F_{(1, 698)} = 5.3$, $P < 0.05$), respectively 6 years after (4 years from the start of the research) mean foliage was significantly lower.

3.3. Hymenoscyphus fraxineus cultures

The pathogenic fungus *H. fraxineus* is present in almost all ash stands in the Czech Republic, so as expected it was also found in all 20 samples collected in the PRP in this study. The branch sample showed clear signs of fungus infestation (dying twig terminals, substitute sprouts, dying in the same growing season, patches of dead bark especially on the bases of substitute sprouts). The sampling was done in late May 2015, somewhat early for full infestation development.

To isolate the fungus, the samples of wood and phloem were collected from the border between the living and dead parts of the sprout from the previous year, and small amounts of the infested material were placed on MEA. The isolated fungus cultures formed phialides and conidia on the mycelium after 13–21 days, typical for the anamorphous stage of *H. fraxineus* Fig. 5.

Cultures of *H. fraxineus* grew relatively slowly in Petri dishes containing MEA; the culture shown in Fig. 5 was photographed after 15 days of growth in the dark at 20-22 °C. The mycelium was initially white, appeared hyaline under the microscope, and later turned grey in some parts. Production of conidia was observed in older cultures (after more than 20 days) on the conidiogenous cells - phialides type *Hymenoscyphus*, which grew individually on the air, slightly off-white mycelium. Morphological featu-

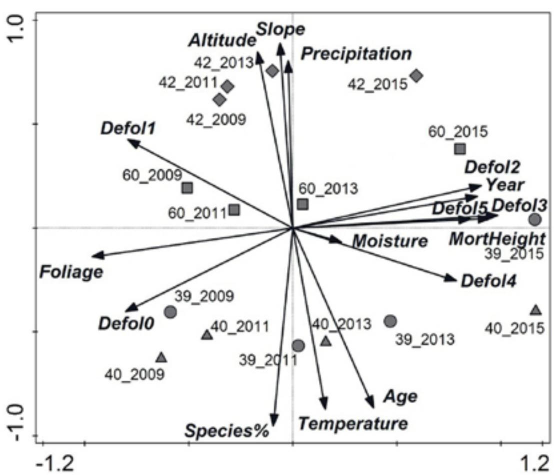

Figure 6: Ordination diagram showing results of PCA analysis of relationships among plot attribute (***Altitude, Slope, Moisture of soil***), climatic data (***Temperature, Precipitation***), stand parameters (***Species %*** percentage ash in stand, ***Age*** mean stand age) and health status (***Foliage*** mean foliage of all trees, ***Defol0-5*** defoliation level, ***MortHeight*** mean height of dead trees); Codes: ●, ▲, ◆, ■ indicate plots with number of year record (2009–2015)

res of isolates from the Krkonoše Mountains were typical for *H. fraxineus* described by Kowalski (2006), specifically conidia 2.5–3.5×2.0 µm, phialides 14–22×3.5–5.0 µm at the base and 2.0–2.5 µm at the collarette.

3.4. Relationships among plot attributes characteristics, stand parameters and health condition

Results of the PCA analysis are presented in Figure 6. The first ordination axis explains 64%, the first two axes together explain 85% and the first four axes together explain 95% of variability in the data. The first axis x represents the mean foliage of trees together with mortality (defoliation level 5) and mean height of dead trees. The second axis y represents plot slope and precipitation with percentage of ash and temperature. Percentage of ash was positively correlated with temperature and stand age, whereas these parameters were negatively correlated with altitude, plot slope and precipitation. These parameters were independent from time. Mortality, the mean height of dead trees (first suppressed and sub-dominant trees dead), defoliation level 2, 3 and 4 increased with time, while mean foliage of all trees, defoliation level 0 and 1 decreased with time. The contribution of soil moisture was small, but the health condition deteriorated faster on plots with wetter sites ($r=0.69$, $P=0.27$). The dynamics of parameters over the 6 years was remarkable especially for PRP 40 and PRP 39 as marks of each record are fairly distant from one another whereas marks for PRP 42 and 60 are relatively close together in the diagram. Plots differed from one another especially in 2015, when PRPs with lower altitude and slope occupied the extreme right part of the diagram, which would be expected with the greater deterioration of health condition and higher mortality of the trees.

4. Discussion

The wide range and rather high representation of European ash might be expected to reduce the seriousness of European ash dieback, but conversely, its wide range and the enormous number of dying trees will probably influence the biodiversity of forest ecosystems across great swathes of Europe (Pautasso et al. 2013a). The situation gets even more complicated as European ash has not only spread in the oak vegetation zone – perhaps owing to the increased nitrogen content from nitrogen deposition (Hofmeister et al. 2004) – but has also colonized unkempt meadows as a pioneer species in plant succession (Marie-Pierre et al. 2006). Furthermore, the species has vigorously spread beyond rural areas, especially in mountainous areas of Europe (Marigo et al. 2000; Střeštík and Šamonil 2006). This extensive expansion means that its dieback has even more far-reaching consequences. If the European ash were less widespread, the damage would not be as grave (Pautasso et al. 2013a). Therefore, it is important that forest researchers identify factors influencing the health and vitality of the European ash to ensure its successful growth, quantity and quality of its wood in various sites and stand conditions (Dobrowolska et al. 2011).

The pathogenic fungus *H. fraxineus* is considered to be the most frequent cause of ash dieback in Europe (Kowalski 2006; Bakys et al. 2009a; Pautasso et al. 2013a). Research indicates that ash dieback is occurring throughout almost all of Europe, from Poland (Kowalski 2006; Orlikowsky et al. 2011) to Denmark (Skovsgaard et al. 2010), Austria (Halmschlager and Kirisits 2008; Kirisits 2011), Slovakia (Adamčíková et al. 2015) and the United Kingdom (Mitchell et al. 2014). Recent investigations have also identified this invasive fungal pathogen on petioles of *Fraxinus mandshurica* in Japan (Zhao et al. 2013) and on *F. mandshurica* and *F. chinensis* in Korea (Gross and Han 2015). *H. fraxineus* will most likely spread within the whole range of European ash (Pautasso et al. 2013a). On a local scale, dead, dying and infected ash trees occur in massive volumes across a wide spectrum of stands and ages of trees (Jönsson and Thor 2012). Some researchers ascertained 70% of dieback in relation to the individual sensitivity and resistance (McKinney et al. 2011; Pliũra et al. 2011). The rest of the population survives probably owing to hereditary resistance mechanisms (Jönsson and Thor 2012) or certain phenologic specifics such as the early senescence of leaves (McKinney et al. 2011).

The dynamics of the stands' health condition for the Krkonoše Mts. indicates a gradual reduction in mean annual foliation of ash of 2.7% in live trees and 4.6% in all trees between 2009 and 2015. In 2012–2015 the rate of defoliation significantly accelerated; the mean annual decrease in foliation reached 3.9% in living trees and 6.3% in all trees. A similar increase in stand damage at this time was observed in ash seed orchards in Germany, where mean defoliation was 3.8% between 2012 and 2013 (Enderle 2015). Slightly smaller annual damage development of crown 2.7% (2011-2013) was observed from ash alluvial alder stands in Lužické hory Mts. (Havrdová 2015). The year 2007 also marked a period of decreased radial growth of attacked trees by *H. fraxineus* on study stands. Annual ring width of infected trees was almost 3 times lower than growth of healthy trees after 7 years. According to standard ring chronology the mean radial growth was significantly lower 2 years after the supposed attack by this fungi; significant deterioration of foliation was observed 4 years later.

Mortality was higher in trees of average or below-average size in the stand, especially in trees with suppressed growth (Skovsgaard et al. 2010). This result also occurred on the sites investigated in our study, especially in the early years of the study, when the fungus infected almost exclusively co-dominant and younger trees. Later, at the peak of the infection, dominant trees began to be affected as well. Havrdová (2015) also confirms the negative correlation of tree height with the occurrence of the disease, but the positive impact of the share proportion of ash was not confirmed in our study due to the small number of plots. According to research in western Hungary, the fungus was also more common in younger ash stands (Koltay 2012). Some authors doubt there are effective measures that would prevent European ash from dying. McKinney et al. (2011) even suggest that ash populations might collapse entirely. Nevertheless, Skovsgaard et al. (2010) propose phytosanitary measures for foresters and recommend that efforts be concentrated primarily on tending young forest stands containing European ash to reduce the mortality. The early years are the crucial growth phase.

In addition, on study PRPs the health condition deteriorated relatively faster with subsequent mortality on the sites more influenced by soil moisture. Similarly, other studies (Havrdová et al. 2014; Havrdová 2015) from alluvial stands of ash in the Czech Republic reported the negative effect of humidity on infection by fungi (the difference in damage of crowns to 14.7%). Koltay (2012) also confirmed from the infested forest stands that the infestation was more frequent on sites with plenty of water, deep soil and frost-hollow. Water is an important factor in ash dieback. Dobrowolska et al. (2008) state that the lowering of the ground water level, repeated deviation/fluctuation of moisture and temperature from usual rates, atmospheric pollution, mechanical injury by animals and other factors may influence the general health condition of individual trees and consequently influence the susceptibility of trees to attacks by *H. fraxineus*. In our results, dieback was first observed on PRPs with lower altitude and flat site. Stands with higher altitude and trees on peaks or homogenous slopes were less affected (Havrdová et al. 2014; Havrdová 2015). Damage from the fungus at elevations above 550 m above sea level is half (average damage of crowns 6.6%) compared to an altitude below 400 m (15.3% of damage; Havrdová 2015). Similarly, in Austria the fungus was first found at eleven locations, only two of which were in the province Upper Austria in contrast to seven in the province Lower Austria and two in the province Vienna (Halmschlager and Kirisits 2008).

Some authors mention also disputable "positive" aspects of the ash dying, albeit short-term, such as the diversity of species that thrive on the dying wood (Pautasso et al. 2013a). Slowly dying, weakened trees host numerous organisms that are scarce in the commercial forests of the Europe (Heilmann-Clausen and Bruun 2013). On the other hand, when an ash population shrinks to a critical level, species reliant on the ash might disappear, such as lichen communities (Jönsson and Thor 2012) whose absence would be detrimental to other animals (Asplund et al. 2010). European ash dieback also provides space for natural clearings, especially on moist and fertile soils, where the ash once prevailed (Heilmann-Clausen and Bruun 2013). This also enhances biodiversity (Robertson et al. 1995) but also might lead to undesirable expansion of non-native tree species such as *Ailanthus altissima* or *Robinia pseudoacacia* (Pautasso et al. 2013b).

In general, massive dieback of tree species in reaction to natural disturbances, including insect pests or fungi, is a phenomenon considered natural in forests of the temperate zone in the Northern Hemisphere by some authors (Heilmann-Clausen and Bruun 2013), but it is necessary to emphasize the fact, this pathogen is highly invasive. In Europe, pathogenic fungi are also responsible for Dutch elm disease (Solla et al. 2005; Hintz et al. 2013), and in North America another pathogenic fungus nearly obliterated populations of American chestnut (Kashian and Witter 2011). In North America, European ash was also severely devastated by a beetle (Siegert et al.

2007). We have to anticipate similar phenomena and take responsible measures in forest management to prevent the damage. Active management recommendations in ash stands with enough healthy trees are selective thinning of trees affected by *H. fraxineus* (Skovsgaard 2013; Longauer 2015), where prospective trees are left for early selection of superior resistant genotypes (McKinney et al. 2011; Lobo et al. 2014). In stands with extensive attacks by the fungal pathogen, it is necessary to replace pure ash stands with a mixed forest (Skovsgaard 2013). Given the current situation, it is now recommended that the proportion of ash trees be gradually reduced in selected affected ash stands. At the same time the share of alternative replacement tree species, such as *Quercus robur, Acer pseudoplatanus, Populus spp.*, and *Prunus padus*, would be increased (Havrdová et al. 2014; Mitchell et al. 2014). Nevertheless, diseases are expected to spread as various global changes accelerate changes in the biodiversity, health and productivity of tree species (Gange et al. 2011).

5. Conclusion

Over 6 years, detected *H. fraxineus* caused defoliation of European ash up to 27%, mortality reached 16% and radial increment decreased to 35% of normal growth. The faster ash dieback was observed on sites with lower altitude, with higher soil moisture and on more flat localities compared to the slopes. Considering that no effective protection has been developed to stop *H. fraxineus*, the only step currently is to introduce preventive measures, such as growing ash in mixed stands with other tree species, improvement felling to support healthy ash trees, providing space for ash trees and removing the infected trees, using all means of legislation, financial support and forest managers motivation. It is worth to favour the ash trees and grow stands as resistant and ecologically stable as possible. It is highly advisable to use the most resistant ecotypes suitable for given macroclimatic and microclimatic conditions. Until researchers can answer the vital questions about how *H. fraxineus* is spread or confirm or disprove the hypothesis of its spreading via abiotic ways (by wind and rain), we can recommend negative selection as the only management action in the affected stands.

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