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Phenotypic variability of *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl under the ash dieback disease in the Czech Republic

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Abstract

Papić S., Buriánek V., Longauer R., Kudláček T., Rozsypálek J. (2018): Phenotypic variability of *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl under the ash dieback disease in the Czech Republic. J. For. Sci., 64: 279–288.

The study was carried out in the experiment with 16 provenances of common ash (*Fraxinus excelsior* Linnaeus) and 2 provenances of narrow-leaved ash (*Fraxinus angustifolia* Vahl) at a series of 5 parallel trial plots established in a gradient from lowland riverine to upland ravine sites. The role of the site, ash species and the provenance of common ash proved to have significant effects on the intensity of ash dieback (ADB) associated with the infection by *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya at the age of 20 years. Narrow-leaved ash was healthier, surviving and growing better than common ash on the trials situated inside as well as beyond its natural range. The ADB intensity was lower in the medium altitude and more easterly located trial plots with a more continental climate. The provenance of forest reproductive material proved to have a significant effect on the ADB damage and survival rate as well as the growth of ash across the trial plots of the experiment.

Keywords: *Hymenoscyphus fraxineus*; common ash; narrow-leaved ash; site; species; provenance effects

Ash dieback (ADB) was first recorded in Poland in the mid-1990s (Kowalski 2006) and since then it has become widespread from the Baltics, through all of Central Europe up to the Atlantic coast of France and the British Isles. It results in a serious damage and mortality of ash trees of all age classes from floodplains up to the mountain forests (Skovsgaard et al. 2010).

The causing agent, *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosaya, is an invasive alien species to Europe originating in East Asia (Zhao et al. 2012; Gross et al. 2014). In the Czech Republic, the ADB was first reported in 2009, although ash decline symptoms were reported many years earlier (Jankovský, Holdenrieder 2009). Typical disease symptoms are wilting of leaves,

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necrotic lesions in the bark and reddish discolorations of branches and stems (Lenz et al. 2016). Its first symptoms are visible on leaves, starting as small necrotic spots which are spreading further and causing necrotic lesions on leaves, twigs, and stems, eventually leading to wilting and dieback of girdled shoots. A characteristic feature is the formation of epicormic shoots which emerge from dormant buds located deeper in a crown due to the dieback of younger shoots. In the later stages of infection, compensatory shoots can appear on the trunk as well, giving a tree a characteristic bushy appearance (McKinney et al. 2014).

According to Kowalski (2006), Kirisits and Cech (2009), Skovsgaard et al. (2010), and Pliūra et al. (2011), the intensity and the progress rate of the dieback depends on the age of host trees, population density, location, microclimate, genetic factors and on the presence of other parasitic and opportunistic organisms.

In relation to the intraspecific variation of common ash, the analysis of the largest to-date provenance experiment performed by Kleinschmit et al. (1996), showed that the site quality had the most significant effect, followed by the effect of provenance and provenance × environment interaction. The ecotypic pattern of variation was observed for flushing and bud set in relation to the provenances from Romania. The significant role of the site and provenance was confirmed by Buriánek et al. (2017), highlighting the potential for selection of suitable provenances and parent of families for different deployment zones.

Many authors reported considerable intraspecific (genetic) variation in the tolerance of common ash to the infection by *H. fraxineus*.

Relative resistance to the disease was identified at the individual and progeny levels (Kjær et al. 2012; McKinney et al. 2012; Stener 2013; Pliūra et al. 2014) as well as with regard to the provenance and source population (Pliūra et al. 2011).

The heritable resistance was conclusively proved by Lobo et al. (2015), which found correlation coefficients at 0.85 between crown damage of mother clones and their progeny, and at 0.72 between crown damage of mother clones and their progeny necrosis after controlled inoculation. The assessments done on half-sib progenies in different years (2009, 2012, 2014) were strongly genetically correlated (0.88–0.91) too (Lobo et al. 2014). The ADB tolerance appears to be of quantitative (polygenic) nature in spite of its strong genetic control (Kjær et al. 2012). However, only 1–5% (Pliūra et al. 2011; Kjær et al. 2012) of current generation trees tolerate infection of *H. fraxineus* enough for their successful survival and reproduction. Although the gradual decline of most ash trees is thus likely to continue, strong genetic control of the infection makes a selection of infection tolerant parent trees, and production of improved planting stock, possible for restitution of ash in forests and landscapes.

With reference to the available knowledge about the role of the host species, site characteristics and genetic factors, the objective of the present study is to analyse effects of ash species, site quality, and provenance of reproductive material on the intensity of ADB.

MATERIAL AND METHODS

Provenance experiment. Our study was carried out in a provenance (common garden) experiment, where reproductive materials representing different source populations (provenances) were planted simultaneously in a series of 11 trial plots in different environments. It allowed studying the effect of a planting site along with the provenance and provenance × site interactions. The experiment was established by the Forestry and Game Management Research Institute (VÚLHM) Jíloviště-Strnady in 1999 and contained 33 provenances of common ash and 2 of narrow-leaved ash at the time of establishment (Buriánek 2000). In each trial site, 3 × 50 seedlings per provenance were planted in a randomized block design with 3 repeats. The spacing of three-year-old seedlings was 1 m in a row and 2 m between rows.

Due to the damage by game browsing, only 5 plots were found suitable for our field study. Two of them are situated in western and three in the eastern part of the country (Fig. 1). They still cover a broad scope of sites from lowland alluvia to the upland ravine and karst forests (Tables 1 and 2).

On all 5 trial plots, 26 provenances of common ash and 2 of narrow-leaved ash are represented. While different subsets of provenances were planted in individual plots, we focused only on provenances represented simultaneously in at least 3 plots in both eastern and western parts of the country. The resulting set of 16 provenances of common ash and 2 provenances of narrow-leaved ash still represents well the territory (Fig. 1) and the broad scope of sites (Tables 1 and 2) where common and narrow-leaved ash occur in the Czech Republic. The largest variation among the locations of provenances in the climatic variables – two-fold – was detected in annual and summer mean precipitation (Tables 1 and 2).

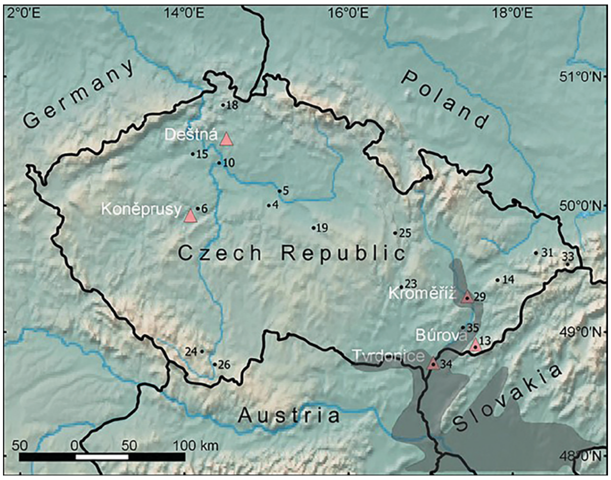

Fig. 1. Location of component trial plots, the location of provenances and local part of the natural range of *Fraxinus angustifolia* Vahl

Field assessment. The ADB damage of trees at individual plots was assessed along with the measurement of their height (using Senshin height measurement pole) from November 2015 until March 2016. The ADB damage was assessed using the protocol developed by Rozsypálek (2015) with focus on the presence of necroses, dead and epicormic branches in the crowns of individual trees, attributable to the infection by *H. fraxineus*. Our approach was similar to the field studies of Pliūra and Baliuckas (2007), Skovsgaard et al. (2009, 2010), Pliūra et al. (2011), and Stener (2013) taking into account the fact that defoliation and necroses, scored in August–September, cannot reflect properly the condition of susceptible, but still vigorous, trees compensating dieback in their crowns resprouting epicormic branches (Enderle et al. 2017). We classified individual trees into 10 classes: (*i*) tree without any visible damage (necroses) or dead peripheral branches, (*ii*) tree with nearly full vitality but with a few necroses and dead peripheral branches, (*iii*) visibly damaged crown with multiple dead peripheral branches and visible epicormic shoots, (*iv*) trees with distorted, half-declined crown, epicormic shoots prevail, (*v*) trees having only secondary crown made mostly of compensatory epicormic shoots, (*vi*) heavily damaged trees with only irregular clusters of epicormic shoots alive in crowns, (*vii*) trees on which only irregular clusters of adventitious stem offshoots are alive, (*viii*) tree showing only residual vitality having only few stem suckers alive, (*ix*) dead trees exhausted by infection of *H. fraxineus*, (*x*) trees which died due to other causes than infection by *H. fraxineus*, mostly root and collar rots and ash bark beetle. Due to unclear relationships of these factors to the ADB, this category was not considered in the mean ADB scores of provenances and further analyses.

Environmental variables. Climatic variables for trial sites and locations of provenances were obtained from the WorldClim database (Hijmans et al. 2005) with 30 arc-second resolution. As independent variables, we considered mean annual air temperature and precipitation as well as temperature and precipitation from April till September corresponding with the vegetation period. With regard to the seasonal biology of *H. fraxineus* and its maximal infection pressure prior to the onset of host leaf senescence (Hietala et al. 2013), we also considered the mean temperature and precipitation of summer months (June–September), warmest month (July mostly), and of September when the infection is manifested by the wilt and premature shedding of leafs. Along with annual means, only climatic variables with significant and nearly significant ($P > 0.90$) effects on biometric charac-

Table 1. Basic information about location, site and climatic conditions of trial plots

Provenance	Longitude	Latitude	Altitude (m a.s.l.)	Site and soil type	Mean annual temperature (°C)	Mean precipitation (mm)			
						annual	VI–IX	VII	IX
Tvrdonice	17°02'31.2"E	48°46'15.6"N	168	alluvial plain, fluvisol	9.4	648	318	93	58
Kroměříž	17°27'25.2"E	49°16'58.8"N	201	alluvial plain, fluvisol	8.6	600	295	82	53
Deštná	14°30'35.1"E	50°31'16.9"N	240	shallow valley, cambisol	8.2	599	331	87	64
Koněprusy	14°02'50.7"E	49°55'29.4"N	417	hill slope NW 15°, limestone ranker	7.2	545	286	87	48
Búrová	17°33'23.0"E	48°53'54.5"N	474	hill slope NW 16°, cambisol (Kag)	7.5	708	257	76	42

Tvrdonice – Forest of the Czech Republic, State Enterprise (FCR), Forest Enterprise Židlochovice; Kroměříž – Archidioecesis Forests and Estates; Deštná – Forest District (FD) Česká Lípa; Koněprusy – FCR FD Nižbor; Búrová – FCR FD Strážnice

Table 2. Basic information about provenances including names of provenances, forest district and local name, coordinates, site type and climatic characteristics

Provenance No.	Provenance name	Latitude	Longitude	Altitude (m a.s.l.)	Site type	T_{ma} (°C)	Mean precipitation (mm) annual	VI–IX	VII	IX
***Fraxinus excelsior* Linnaeus**										
4	SFE Kostelec nad Černými lesy – Svojšice	50°00'21.6''N	15°02'31.2''E	265	riverine	8.6	493	245	73	39
5	FD Nymburk – Libice	50°07'12.0''N	15°10'19.2''E	190	riverine	8.9	483	243	75	37
6	FD Nižbor – Karlštejn	49°56'16.8''N	14°11'02.4''E	320	karstic	8.5	513	245	72	40
10	Mělník (Lobkowiczké lesy) – Úpor	50°19'55.2''N	14°29'16.8''E	160	riverine	8.9	526	258	78	43
13	FD Strážnice – Javorník	48°52'01.2''N	17°32'24.0''E	380	alluvial-hill	8.5	681	320	85	62
14	FD Bystřice pod Hostýnem – Rajnochovice	49°25'15.6''N	17°48'46.8''E	580	scree	7.3	751	360	94	67
15	FD Litoměrice – Budyně nad Ohří	50°24'18.0''N	14°07'12.0''E	180	riverine	8.9	556	264	78	45
18	FD Česká Lípa – Prysk	50°00'39.6''N	13°55'19.2''E	500	scree	7.0	716	318	96	57
19	FD Ronov nad Doubravou – Běstvina	49°50'06.0''N	15°34'58.8''E	320	scree	8.2	499	249	74	40
23	SFE Křtiny – Bílovice nad Svitavou	49°16'55.2''N	16°38'09.6''E	500	karstic	7.8	553	266	75	47
24	FD Český Krumlov – Chvalšiny	48°51'39.6''N	14°14'24.0''E	720	scree	6.9	925	382	114	72
25	FD Svitavy – Nová Ves	49°47'24.0''N	16°34'22.8''E	560	scree	6.7	606	303	85	51
26	FD Kaplice – Silniční domky	48°45'10.8''N	14°23'09.6''E	800	scree	6.5	948	396	121	75
29	FD Bystřice pod Hostýnem – Kroměříž	49°17'13.2''N	17°26'42.0''E	200	riverine	9	599	295	82	53
31	FD Frenštát pod Radhoštěm – Palkovské hůrky	49°37'40.8''N	18°16'40.8''E	430	scree	7.7	804	386	104	72
33	FD Jablunkov – Mionší	49°32'31.2''N	18°39'57.6''E	720	scree	5.4	1,050	474	124	89
***Fraxinus angustifolia* Vahl**										
34	FE Židlochovice – Tvrdonice	48°44'45.6''N	17°00'54.0''E	160	riverine	9.7	653	318	93	58
35	FD Strážnice – Nedakonice	49°01'01.2''N	17°22'51.6''E	175	riverine	9.3	614	301	83	57

SFE – School Forest Enterprise, FD – Forest District, FE – Forest Enterprise, T_{ma} – mean annual temperature

teristics are included in Tables 3 and 4. We also calculated Conrad continentality index – *k* (Conrad 1946) using Eq.1:

$$k = 1.7A/\sin(\varphi + 10) - 14 \quad (1)$$

where:
A – difference between the mean temperature (°C) of the warmest and coldest month,
φ – latitude (in radians) of the place in question.

Data analysis. Differences in height and ADB damage scores attributable to the species, trial sites and provenances were tested by analysis of variance using the GLM procedure of the statistical system SAS (Version 9.1, 2010); species, provenance, and trial site were considered fixed-effect factors. The following model (Eq. 2) was used for the tree height and ADB score as response variables:

$$Y_{ijkl} = \mu + \text{species}_i + \text{prov}(\text{species})_{ij} + \text{plot}_k + (\text{spec} \times \text{plot})_{ik} + (\text{prov} \times \text{plot})_{jk} + \text{block}(\text{plot})_{lk} + \varepsilon_{ijklm} \quad (2)$$

where:
Y_{ijkl} – ADB score (or height) of m^{th} tree of the i^{th} species and j^{th} provenance growing on k^{th} plot within l^{th} block,
μ – grand mean,
plot_k – effect of k^{th} plot,
ε_{ijklm} – residual error,
species_i – effect of i^{th} species,
$\text{prov(species)}_{ij}$ – effect of j^{th} provenance within i^{th} species,
$(\text{spec} \times \text{plot})_{ik}$ – effect of interaction between species and plot,
$(\text{prov} \times \text{plot})_{jk}$ – effect of interaction between provenance and plot,
block(plot)_{lk} – effect of lth block within k^{th} plot.

For the identification of general provenance-related trends, average ADB scores and heights of provenances were correlated with geographic coordinates and climatic variables of the locations of their origin. Because the experiment was unbalanced, least-square means of mean tree height

Table 3. ANOVA of the ash dieback (ADB) scores and tree heights ($P < 0.001$)

Source of variation	ADB score		Tree height,
	df	*F*-test	*F*-test
Species	1	423.6	637.6
Provenance (species)	16	6.6	35.6
Trial plot	4	429.8	533.9
Species × plot	3	8.1	12.5
Provenance × plot	48	3.6	2.73
Block (plot)	9	3.6	20.21
Error	2,905		

df – degree of freedom

ADB score calculated using the procedure GLM were used to measure the performance of individual provenances and Spearman's rank correlation coefficients were calculated for individual provenances.

Joint-regression analysis (Finlay, Wilkinson 1963) was used to assess the stability of individual provenances represented in at least 3 trial plots. Their performance in terms of the ADB score or tree height was regressed against the mean performance of all provenances for each trial plot whereas the slopes of linear regression described the stability. The significance of each regression slope was tested using the one sample *t*-test. Results of *t*-tests for the null hypothesis H_0 when $b = 1$ are rather approximate, however, since regression coefficients are normally distributed only around $b = 0$.

RESULTS

Species, trial sites, and provenances exhibited a significant effect on ADB scores and tree heights (Table 3). Nevertheless, the provenance × trial site interaction was also significant both for ADB score and height, meaning that the provenances differ in their reactions to the environments of the trial sites.

Species and site effects

In general, narrow-leaved ash clearly outperformed common ash at all sites where the two species were planted together. It holds true for all response variables, including the survival rates, ADB scores and tree heights (Table 4).

Regarding the effect of site, ADB damage was found to be considerably higher on trial plots Deštná and Koněprusy situated in the western part of the Czech Republic. It can be explained by a generally more advanced ADB and location of the trail plot Deštná in a climatic region with lower annual insolation, lower summer-winter amplitudes and higher ambient humidity in comparison with other trial sites. The trial plot Koněprusy, on the other hand, was established on a limestone ridge with shallow scree soil resulting in a retarded growth and mostly recent mortality attributable to the drought rather than the effect of infection by *H. fraxineus*.

In general, narrow-leaved ash clearly outperformed common ash on all sites where the two species were planted together. It holds true for all response variables, including the survival rates, ADB scores and tree heights (Table 4) at trial plots Tvrdonice and Kroměříž which are inside as well as Deštná and Búrová which are clearly outside the natural range of narrow-leaved ash, which includes only the alluvial plain of Morava river in the Southeast Czech Republic (Fig. 1) (Úradníček, Maděra 2001).

Provenance effects

Provenance effects, if detected, represent the part of genetic (within-species) variation attributable to the adaptation of local populations to different site conditions. In our study, we analysed them particularly in relation to the intensity of infection

Table 4. Survival, mean ash dieback (ADB) score and mean height of ash trees in individual trial plots at the age of 20 years. Underlying site and species effects were significant in all cases

Trial plot	*Fraxinus excelsior* Linnaeus				*Fraxinus angustifolia* Vahl			
	No. of planted trees	Survival rate (%)	ADB score	Mean height (m)	No. of planted trees	Survival rate (%)	Mean infection score	Mean height (m)
Tvrdonice	2,100	19	4.02	6.86	300	52	3.61	10.07
Kroměříž	2,400	26	4.08	8.31	300	39	2.97	9.48
Búrová	2,200	21	4.05	10.33	300	53	2.90	11.63
Deštná	2,550	21	7.41	6.09	150	35	5.38	7.08
Koněprusy	1,500	35	5.83	1.67	–	–	–	–
Across plots	10,750	27.2	5.085	7.51	1,050	44.75	3.708	9.71

Table 5. Mean ash dieback scores of provenances across all plots with provenances ordered into homogenous groups (marked by the same letter) by the Duncan test

Provenance No.	Provenance name	No. of trees	ADB score	Duncan grouping			
***Fraxinus angustifolia* Vahl**							
35	FD Strážnice – Nedakonice	185	3.28		A		
34	FE Židlochovice – Tvrdonice	247	3.66		A		
***Fraxinus excelsior* Linnaeus**							
23	SFE Křtiny – Bílovice nad Svitavou	67	4.40			B	
10	Mělník (Lobkowiczké lesy) – Úpor	152	4.74		C	B	
25	FD Svitavy – Nová Ves	288	5.13		C	B	D
5	FD Nymburk – Libice	129	5.23		C		D
31	FD Frenštát pod Radhoštěm – Palkovské hůrky	100	5.44	E	C		D
29	Archidioecesis Forests and Estates – Kroměříž	108	5.46	E	C		D
14	FD Bystřice pod Hostýnem – Rajnochovice	162	5.48	E	C		D
33	FD Jablunkov – Mionší	173	5.61	E			D
13	FD Strážnice – Javorník	152	5.61	E			D
6	FD Nižbor – Karlštejn	91	5.68	E			D
24	FD Český Krumlov – Chvalšiny	180	5.76	E			D
26	FD Kaplice – Silniční domky	170	5.77	E			D
15	FD Litoměrice – Budyně nad Ohří	228	5.83	E			D
19	FD Ronov nad Doubravou – Běstvina	162	5.98	E	F		D
4	SFE Kostelec nad Černými lesy – Svojšice	89	5.98	E	F		
7	FD Křivoklát – Pustá Seč	55	6.25		F		

FD – Forest District, FE – Forest Enterprise, SFE – School Forest Enterprise

by *H. fraxineus*, considering the survival rate and growth as complementary characteristics.

Performance of individual provenances was characterized by means of the survival rate, mean ADB score, and mean height of surviving trees. Significant differences between provenances were detected in each variable at any of the plots. Table 5 provides bulk information about the ADB scores of provenances across all plots together. Provenances of common ash (16) revealed considerable, yet statistically significant, differences in their health condition. When the ADB scores at individual trial plots shown in Table 6 were considered (data not shown), differentiation of provenances was bigger at the lowland alluvial plots (Tvrdonice, Kroměříž) than in the northwest (Deštná, Koněprusy) of the Czech Republic. Two narrow-leaved ash provenances revealed only minor mutual difference on the plots where they were planted together. Their overall difference is caused by the sole presence of provenance 34 on the plot Deštná, where overall ADB score is much higher than elsewhere as seen in Table 6.

Table 6 provides plot-wise mean ADB scores of provenances. In common ash, provenances representing putative riverine (provenance: 4, 5, 10, 13, 15, 29) and upland (provenance: 6, 14, 18, 19, 23, 24, 25, 26, 31, 33) ecotype did not reveal a specific response pattern among the plots. No riverine provenance revealed, however, at least marginally significant difference ($P < 0.10$) from one in the ADB score (Table 6, last column). Their average stability (Finlay, Wilkinson 1963) means that they follow general ADB trend along the site gradient including alluvial plain (Tvrdonice, Kroměříž), upland alluvium (Deštná), hillslope (Búrová) and karstic (Koněprusy) sites. Of the two provenances from limestone parent rocks (6, 23), local provenance 6 was of the healthiest ones in Koněprusy situated in the Czech Karst.

Most reliable conclusions, about provenance × site interaction can be drawn only for provenances represented at all trial plots, however. Two of them, No. 19 and 24, revealed significant positive response demonstrated in their improving health towards trial plots with lower overall ADB score.

We also found a marginally significant ($r = -0.31$, $P > 90\%$) negative correlation between the ADB scores (ordered from 1 – no infection to 9 – dead) and the mean heights of provenances. Provenances less prone to infection and healthier trees in general, thus grow better than susceptible ones (Table 7). Another marginally significant negative correlation of ADB scores and survival rates ($r = -0.36$) indicates better survival of less infected provenances. Its marginality suggests, however, that survival rates

Table 6. Plot-wise mean ash dieback scores of provenances and their stability coefficients assessed using joint regression analysis

Provenance		Trial plot					Coefficient		
No.	name	Tvrdonice	Kroměříž	Búrová	Deštná	Koněprusy	*b*	adjusted R^2	*t*
***Fraxinus excelsior* Linnaeus**									
4	Kostelec nad Černými lesy – Svojšice	4.71		4.20	7.38	5.71	0.736	0.757	–0.89
5	Nymburk – Libice	3.04	3.22	3.91	7.58		1.205	0.97	1.37
6	Nižbor – Karlštejn		3.70	3.77	7.51	5.04	1.05	0.874	0.18
7	Křivoklát – Pustá Seč			5.00	6.82	4.91	0.525	0.842	–2.09**
10	Mělník – Úpor	3.91	3.46	4.22	6.74		0.815	0.929	–1.18
13	Strážnice – Javorník	3.82	3.68	3.80	7.79		1.149	0.987	1.59
14	Bystřice pod Hostýnem – Rajnochovice	4.31	4.41	4.08	7.43		0.907	0.988	–1.33
15	Litoměřice – Budyně nad Ohří	4.07	4.28	4.64	7.20	6.83	0.903	0.895	–0.54
19	Ronov nad Doubravou – Běstvina	3.26	4.09	3.65	8.31	5.74	1.318	0.986	3.53*
23	Křtiny – Bílovice nad Svitavou		4.43	4.20		6.09	0.353	0.991	–19.61*
24	Český Krumlov – Chvalšiny	4.13	4.17	4.31	7.93	6.13	1.101	0.991	1.66**
25	Svitavy – Nová Ves	3.46	3.80	3.94	6.35	6.31	0.837	0.849	–0.80
26	Kaplice – Silniční domky	4.36	4.41	4.05	7.61	6.13	0.978	0.974	–0.24
29	Kroměříž		4.51	3.75	7.26		0.935	0.973	–0.41
31	Frenštát pod Radhoštěm – Palkovské hůrky	4.44	3.32	3.82	8.08		1.185	0.912	0.71
33	Jablunkov – Mionší	4.13	4.37	3.79	7.60		1.001	0.982	0.01
***Fraxinus angustifolia* Vahl**									
34	Židlochovice – Tvrdonice	2.88	3.50	2.62	5.38		0.692	0.936	–2.43*
35	Strážnice – Nedakonice	3.06	3.72	3.12			1.708	0.753	0.73

scores: from 1 – no infection to 9 – dead, *b* – stability coefficient, slope of the regression $y = a + b\bar{y}$, *t* – *t*-test of the null hypothesis H_0: $\beta = 1$, provenance × site interaction: *significant at $P > 95\%$, **marginally significant at $P > 90\%$

Table 7. Survival rates (provided for reference) and mean heights of provenances across all plots with provenances ordered and grouped into homogenous groups (marked by the same letter) according to their means height by the Duncan test

Provenance		Survival rate	Mean height (m)	Duncan grouping for height			
No.	name						
***Fraxinus angustifolia* Vahl**							
35	Strážnice – Nedakonice	0.478	10.98			A	
34	Židlochovice – Tvrdonice	0.485	8.90		B		
***Fraxinus excelsior* Linnaeus**							
5	Nymburk – Libice	0.207	8.67		B		C
29	Kroměříž	0.265	8.57		B		C
13	Strážnice – Javorník	0.253	7.83		D	E	C
31	Frenštát pod Radhoštěm – Palkovské hůrky	0.162	7.74		D	E	C
10	Mělník – Úpor	0.271	7.43		D	E	C
14	Bystřice pod Hostýnem – Rajnochovice	0.262	7.32	F	D	E	C
33	Jablunkov – Mionší	0.276	7.13	F		E	G
15	Litoměřice – Budyně nad Ohří	0.329	6.02	F	H		G
25	Svitavy – Nová Ves	0.414	5.96	F	H		G
26	Kaplice – Silniční domky	0.237	5.81		H		G
24	Český Krumlov – Chvalšiny	0.264	5.40		H		I
19	Ronov nad Doubravou – Běstvina	0.246	5.16		H		I
6	Nižbor – Karlštejn	0.325	5.09		H		I
4	Kostelec nad Černými lesy – Svojšice	0.282	4.97				I
23	Křtiny – Bílovice nad Svitavou	0.388	4.85				I
7	Křivoklát – Pustá Seč	0.260	4.40				I

Table 8. Spearman rank correlation coefficients between phenotypic characteristics of common ash provenances (survival rate, mean height and ash dieback (ADB) score) and descriptors (geographic coordinates, climatic characteristics) of the locations of their origin

Locations descriptors		ADB score	Survival rate	Mean height
Altitude		0.035	0.067	–0.211
Latitude		0.006	0.124	–0.069
Longitude		–0.284**	–0.118	0.548*
Temperature	annual	–0.027	–0.093	0.136
	April–September	–0.010	–0.081	0.137
	June–September	–0.010	–0.077	0.150
	July	0.019	–0.071	0.144
	September	–0.063	–0.042	0.147
Conrad continentality index		0.246	0.179	0.229
Precipitation	annual	0.144	–0.269	0.136
	June–September	0.083	–0.274	0.242
	July	0.130	–0.306*	0.154
	September	0.079	–0.277	0.244

**P > 90%, *P > 95%

can be used rather as an indirect characteristic of ADB damage. Negative, yet significant, correlation (r = –0.463, P > 95%) of survival rates and mean heights of provenances can be explained by a better growth of surviving less infected trees. Surviving trees may grow better thanks to more space available also in more susceptible provenances, however.

Ash dieback score and mean height of provenances seem to be influenced by the geographic origin and site conditions prevailing in their source populations (Table 8). Longitude was found weakly correlated with the ADB scores while moderately yet significantly correlated with the mean height of provenances. It indicates better health and especially the growth of provenances from easterly parts of the Czech Republic. Regarding the climate, weak yet consistent precipitation effects suggest better survival of provenances originating from sites with higher July precipitation amounts. In the ADB coefficient, however, provenance data bulked across all 5 plots did not reveal any similar relationship.

DISCUSSION AND CONCLUSIONS

Role of ash species

F. angustifolia showed considerably higher tolerance to *H. fraxineus* infection (much lower ADB scores and much higher survival rate) at all 4 trial plots where it was planted. Two plots are situated within its natural range, but 2 plots clearly outside of it. Lower susceptibility of *F. angustifolia* to *H. fraxineus* was reported also by Havrdová et al. (2016), Schwanda and Kirisits (2016) and Buriánek et al. (2017). Kirisits et al. (2010) confirmed that *F. angustifolia* is susceptible to the ADB fungus too, and Hauptman et al. (2016) reported significant differences in the tolerance to the ADB among *F. angustifolia* clones based upon the crown damage assessment, inoculation trials, and leaf phenology. Large-scale test with more provenances of *F. angustifolia* representing different parts of the species' natural range could generate more information about ADB tolerance, adaptability, and growth traits in order to close the current knowledge gap about the species at the northern margin of its distribution.

Effect of a site

There are numerous scientific papers studying the effects of site on the severity of ADB. In the Czech Republic, the effect of the site has been confirmed by Havrdová et al. (2016). The existence of adaptation of local ash populations to different site conditions – manifested in the putative highland and riverine ecotype of common ash (Úradníček, Maděra 2001) – makes a precise study of site effect possible only by means of multi-site experiments established using the same sets of clones, progenies or provenances planted in different site conditions. In the present study, different sets of provenances were represented in individual trial plots. Therefore, only provenances represented in at least 3 of 5 plots were included in the analysis. In a narrow sense, however, only 5 provenances planted simultaneously at all 5 trial plots can provide reliable information about the site effects and eventual trends. Regarding the effect of site on ADB, more suitable sites with healthier common ash are in the lower (Kroměříž) to medium (Búrová) elevation in the eastern part of the Czech Republic. This finding needs to be taken with caution because of the influence of local factors such as the quality of soils and climate.

Provenance effects

We confirmed the importance of source population (provenance) of forest reproductive material in the experiment, which was manifested by the significant provenance components in the variance analysis, and by the regressions of phenotypic characteristics of provenances with geographic coordinates and climatic variables of the locations of their origin. The bulk analysis across 5 experimental plots of the experiment does not support the earlier conclusion of Havrdová et al. (2016), drawn from the single plot Deštná, which found less infection by *H. fraxineus* in higher altitude (mountain) provenances of common ash. Instead, the longitude of source population – i.e. the origin reproductive material in the East-West direction – was found important in relation to the growth and ADB intensity of the Czech provenances of common ash.

So far, there are no effective treatments known to cure or mitigate ADB. Our results suggest, on the other hand, the prospects of selective breeding and its potential to mitigate the effects of ADB. Although the most important amount of genetic variation related to the ADB tolerance is at the level of clones (Pliūra et al. 2014) and within families (Pliūra, Baliuckas 2007), sources of more dieback-tolerant forest reproductive material can be found also at the level of provenances. Pliūra et al. (2011), for instance, reported 2-fold (in an extreme case 4-fold) difference in the survival rate and 50% difference in the health score among common ash provenances from Ireland, France, Belgium, Germany, Czech Republic and Lithuania. The survival rate and health condition of local Lithuanian provenances was 50 to 70% better in comparison with those from Central and Western Europe. Although Kjær et al. (2012) did not reveal significant ADB-related provenance effects in a geographically limited area of Denmark, the results of Havrdová et al. (2016) support our conclusions about the important role of the provenance of common ash in the Czech Republic.

With reference to the fact that generally only 1–5% of current generation ash trees are expected to be dieback-tolerant, their identification, conservation and use are utmost important. In the Czech Republic, ash populations are influenced by historical forest fragmentation, shifting tree species composition, and forest regeneration using progenies of few seed parents. Such situation provides a strong argument for targeted selection and use of dieback-tolerating individuals ex situ by means of generative reproductive plantations (seed stands), seedling and clonal seed orchards. The latter, for instance, are capable of generating progenies with improved tolerance in a reasonable time of 7 to 10 years for the ash restoration. Due to the long-term reliance of local forestry on artificial forest regeneration, groups of naturally regenerated noble hardwoods usually occur scattered in conventionally managed forests and, in addition, subject to intensive browsing by overpopulated deer. Perhaps only some gene reserve forests can provide for efficient in situ conservation where gene conservation can be combined with promotion of nature-conforming silvicultural practices.

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