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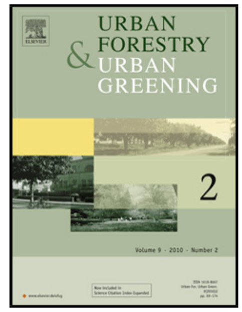

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Title: Survey of *Hymenoscyphus fraxineus* in a central European urban area and exploration of its possible environmental drivers

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Abstract

There are relatively few studies surveying how meteorological and qualitative characteristics of tree sites in urban areas influence *Hymenoscyphus fraxineus* (T. Kowalski) Baral et al. (≡*Chalara fraxinea* T. Kowalski, =*Hymenoscyphus pseudoalbidus* Queloz et al.) or infection by that. Based on the significance of ash trees in urban areas and the frequent use of ash species and their varieties, it becomes increasingly important to identify factors that drive the pathogen. The aim of this study was to identify the effects of both meteorological parameters and tree characteristics on the severity of the disease in the city of Leipzig, Germany. We assessed 401 ash trees along streets and in green spaces distributed across Leipzig. The assessment included scoring the level of damage by *H. fraxineus*, visually estimating the degree of soil sealing under a tree's crown, measuring tree height, the height of crown base and the diameter at breast height, determining sex, year of planting and species variety, as well as recording qualitative characteristics (e.g. secondary pathogens, feeding marks). Mean relative air humidity, mean annual temperature and a combined index of meteorological conditions were used as meteorological parameters. In addition, we sampled tissue of 45 ash trees for the purpose of genetically verifying the occurrence of *H. fraxineus*. We analysed the effects of all variables on ash dieback by using linear mixed effect models. Results show an extensive damage by *H. fraxineus* in the whole city area with 70% of trees being infected. Mean relative air humidity and the index of meteorological conditions show a significant positive influence on the severity of ash dieback, whereas year of planting has a negative effect. Trees with feeding marks at the trunk tend to be more damaged by the fungus. Moreover, we found a significant difference in susceptibility between *F. excelsior* and its variety 'Atlas'. Both meteorological conditions and the susceptibility of species and their varieties should be taken into account when planting ash trees in urban areas.

Keywords: ash dieback, urban trees, urban area, Europe, *Hymenoscyphus fraxineus*, *Fraxinus excelsior*

Introduction

Fraxinus excelsior Linné (1753) is widely distributed in Europe and besides a high economic und ecological value in silviculture, the tree species is frequently planted in urban areas (Roloff and Pietzarka, 1997). Its wide ecological amplitude with respect to water availability and its light crown make it potentially suitable for usage in urban areas (Roloff, 2013).

However, in recent years, *F. excelsior* has increasingly been infected by the ascomycete *Hymenoscyphus fraxineus* (T. Kowalski) Baral et al. (≡*Chalara fraxinea* T. Kowalski, =*Hymenoscyphus pseudoalbidus* Queloz et al.) (Pautasso et al., 2013). In Europe, ash dieback was first recorded in Poland in the 1990s (Przybył, 2002) and since then symptoms have been observed over large parts of the continent across a range of climatic conditions (Husson et al., 2011). While *Fraxinus angustifolia* Vahl (1804) is similar in susceptibility (Lohrer, 2014; Kirisits et al., 2009), the third native species in Europe *Fraxinus ornus* Linné (1753) seems to be least susceptible, with infection being constrained to ash leaves (Schwanda and Kirisits, 2016). The fungus also affects some varieties of *F. excelsior* like *F. excelsior* 'Westhof's Glorie', 'Raywood' and 'Atlas' (Schumacher et al., 2008; Junker, 2013).

Depending on the level of infection, the pathogen causes different symptoms in canopies of ash trees, i.e. wilting of leaves, dieback of shoots, necrotic lesions in bark and can result in the death of trees (Pautasso et al., 2013) (Fig. 1).

Fig. 1

Wind-dispersed ascospores play a primary role in the life cycle of *H. fraxineus*. Produced in apothecia on previous year's dead pseudosclerotical rachises in leaf litter of common ash, they are dispersed by wind from June to September (Timmermann et al., 2011, Cleary et al., 2013). Infectious ascospores then first penetrate green ash leaves followed by rachis up to woody tissue (Cleary et al., 2013; Gross et al., 2014). This spread of mycelia inside the tree's tissues results in the typical symptoms of ash dieback listed above (Bakys et al., 2009). The life cycle of *H. fraxineus* closes when infected leaves or rachises fall to the ground where the fungus again develops pseudosclerotical layers (Gross and Holdenrieder, 2013).

Previous studies were carried out in forests, plantations or in-vitro, providing suggestions but no detailed evidence of the processes and factors influencing the fungus in urban areas. Environmental conditions vary a lot among urban and non-urban areas. Special conditions in climate, especially changes in temperature and air humidity, but also changes in sealing and compression of surfaces, gritting salt, traffic exhausts and pruning render urban areas much more stressful than others (Roloff, 2013). Especially along roads, a number of abiotic factors may affect tree health (Chacalo et al., 2002). Although trees might face increased levels of stress when growing by the roadside, an increase in soil sealing under a tree's crown might negatively affect the life cycle of the fungus, as accumulation of leaves is reduced (Kirisits et al., 2012; Kowalski and Kehr, 2016). In addition, leaves of roadside trees dry off faster due to harsher abiotic conditions. This gives *H. fraxineus* less time, and in consequence less chance, to grow through petiole into shoots (Gross et al., 2014).

We therefore investigated the occurrence of *H. fraxineus* and factors driving the severity of ash dieback in the city of Leipzig, Germany where ash dieback was detected in central riverside forest in 2011 (Leipzig, 2013). We posed the following questions and hypotheses:

1. How often does ash dieback occur in Leipzig?
2. How do mean relative air humidity and mean annual temperature affect the occurrence of *H. fraxineus*? Is it possible to associate these correlations to the combined index of meteorological conditions of Leipzig as classified by the city of Leipzig (Stadt Leipzig, Amt für Umweltschutz, 2010)?
   - Hypothesis a. Ash dieback is more frequent at humid sites.
   - Hypothesis b. Occurrence of *H. fraxineus* decreases with increasing mean annual temperature.
   - Hypothesis c. Based on Hypothesis a and b, the occurrence of *H. fraxineus* can be associated with the combined index of meteorological conditions.
3. How does soil sealing affect the occurrence of *H. fraxineus*? Is there a difference between sites (roadside vs. green space)?

Hypothesis d. Occurrence of *H. fraxineus* decreases with increasing amount of sealed soil under the crown of ashes.

Hypothesis e. Level of damage is higher in urban green spaces than along streets.

4. Is there a difference in the level of damage between *F. excelsior* and its varieties?

Hypothesis f. Varieties differ in the level of damage by the pathogen.

Materials and Methods

Study area

Leipzig (51° 20' 22.904" N, 12° 22' 23.069" E) is situated in the east of Germany in a lowland in north-eastern Saxony where the rivers Pleiße, Elster and Parthe converge (Stadt Leipzig, 2016b, d). In 2016 mean annual air temperature was 10.4 °C and annual precipitation was 469 mm (Stadt Leipzig, 2016c).

Many parks shape the townscape of Leipzig. The city centre is traversed by an alluvial forest of almost 5,900 ha (Enedas e.V:, 2010). The city covers 29,739 km^2 and is divided into ten municipalities (Stadt Leipzig, 2016c).

With 10.7 per cent, *F. excelsior* is one of the main street tree species used in Leipzig, besides *Tilia* sp., *Acer* sp. and *Platanus* sp. (Stadt Leipzig, 2016a).

Ash species

We sampled ash species and varieties based on the tree register of Leipzig. By 2016, the register comprised all street trees and half of the trees located within green spaces as well as geographic coordinates of the trees' location and information on the year of planting. Into our analyses, we included those ash species and varieties, which occurred with ≥ 300 individuals across the city. Subsequently, we created a random sample of 30 trees per municipality, with 15 trees each along roads and in parks by applying stratified random sampling. As there were less trees belonging to one of *F. excelsior*'s varieties than *F. excelsior* itself, and thus fewer individuals per municipality, we created a random sample of 7 trees per municipality for the comparison of *F. excelsior* and its varieties.

This was realised in RStudio (version 1.0.136, © 2009-2016 RStudio, Inc.). This is an interface that can be used for working with R, a language for statistical computing.

Data collection

Examination of selected trees took place in September and October 2016. Trees were assessed from the ground. Height and height of crown base were measured with a Vertex VI (Haglöf, Sweden); diameter at breast height was measured with a measuring tape for circumference at 1.30 m above ground. Furthermore, we recorded the sex of the tree, degree of damage by ash dieback and percentage of leaf fall. We also noted whether the loss of leaves was > 50 %, whether young trees lost their leading shoot and whether secondary damages (fungi, bark beetles, trunk necroses), or features like colouring of leaves, dead wood or sunburn occurred. These as well as soil sealing under crowns of ashes were estimated visually. Additional, we collected tissue of infected ash trees (leaves with rachises and/or shoots) in order to verify the occurrence of *H. fraxineus* on the basis of genetic analyses. Possible sanitary cuttings were recorded and the reason for cutting was checked with the office of municipal affairs (Amt für Stadtgrün und Gewässer).

<u>Degree of damage</u>

A six-point scale developed by "Nordwestdeutsche Forstliche Versuchsanstalt" was used to assess damage by *H. fraxineus* (NW-FVA Abt. Waldschutz, 2013). Additionally, a scale for leaf loss developed by "Bayerische Versuchsanstalt für Wald- und Forstwirtschaft" was used (Tab. 1) (Lenz et al., 2012).

Tab. 1

<u>Sealing under crown</u>

To evaluate the amount of sealed soil under each tree crown, we visually estimated what proportion of the ground was covered by the tree's crown (the figurative of the crown in a square). Sealing was defined and modified as per Haase and Nuissl (2007) with proportion of sealed soil divided in categories of ≤ 20 %, 21 to 40 %, 41 to 60 %, 61 to 80 % and ≥ 81 %.

<u>Sampling of ash tissue</u>

At single ash trees (n= 45; distributed across all municipalities of the city of Leipzig), we sampled plant tissue (leaves with rachises and/or shoots). This was used for verifying the occurrence of *Hymenoscyphus fraxineus* by DNA-analysis and therefore to evaluate the work of the assessor.
Collection took place if there were rachises or leaves with brownish discolouration or necrotic shoots. Tissue was collected in airproof bags. Some trees didn't show symptoms of ash dieback in the crown but on epicormic shoots at the stem base. Therefore, we also took samples for trees with level of damage = 0.

Meteorological Data

Interpolated grid data of mean relative air humidity and of mean annual temperature were provided by "Regionales Klimainformationssystem (ReKIS)" (Retrieved from: http://141.30.160.224/fdm/index.jsp?k=rekis). ReKIS offers an interpolation tool where data can be generated according to criteria defined by the user. We chose data for the time period 1st January 1971 to 31th December 2015 with a constant number of measuring stations (mean annual temperature= 10 measuring stations, relative air humidity= 13 measuring stations), a Gauss-Krüger projection as coordinate system as well as a spatial resolution of 1000 m. This yielded values of mean relative air humidity and of mean annual temperature per grid cell (1000m x 1000m).
In addition, the Environmental Protection Agency of Leipzig provided data on a combined index of meteorological conditions and water areas in the form of vector data. The classification of this is based on local and regional climate measurements in the city of Leipzig. Beside climate data (based on 7 measuring stations) comparable to ReKIS (see above), this index includes measurements of surface temperature, which were performed with an aerial thermal scanner on 22nd and 23rd September 2010. Further measurements of air temperature and air humidity on the ground were performed at 9 different locations in the city area from 20th to 26th September 2010 and by mobile measurements on 22nd and 23rd September 2010 (Leipzig, 2013). Specific landscape data (topography, roughness of surfaces, land use), also affecting local climate, is considered additionally. The index divides Leipzig into different climate areas (Appendix A).

For the survey we used the interpolated data of ReKIS and areas of the combined meteorological index, which are characterised by either cold air or by overheating as well as by areas of water (see Appendix A).

Preparation of data

Sampling and analyses of maps

For analyses of maps we used QGIS (QGIS-version 2.8.9-Wien) and with that we first converted grid data to vector data. Subsequently, we compared climate data with trees by position. Moreover, we created a buffer of 400 m around each water area. This was saved in a separate file and also compared with trees by position.

Genetic analyses

To verify the occurrence of *H. fraxineus*, we first isolated DNA from sampled ash tissue (DNA extraction) using Invisorb ® Spin Plant Mini Kit of Stratec. We used gel electrophoresis to ensure that extraction was successful. Once extracted, we used DNA for standard PCR and a subsequent analysis of DNA fragments. For standard PCR, TopTaq Master Mix Kit of Qiagen with fungal-specific primers Hym_f (5'-AGCTGGGGAAACCTGACTG-3') and Hym_r (5'-ACACCGCAAGGACCCTATC-3') was used (Johansson et al., 2010). These primers are derived from the ribosomal ITS region of *C. fraxinea* (Stenström and Ihrmark, 2005). To verify results of analysis of DNA fragments, six DNA samples were sent to Eurofins (https://www.eurofins.de/genomic-services/) for further DNA sequencing (BLAST-analysis).

Statistical analyses

To analyse whether the pathogen is influenced by the chosen variables, we applied linear mixed effect models using the 'nlme' package for R (Pinheiro et al., 2017) with a significance level of 0.05. We analysed the influence of site (location along streets or in green spaces), height of crown, year of planting, combined meteorological index ('climate area'), closeness to water areas, sex, feeding marks of bark beetles, sealing and mean relative air humidity as fixed factors on the severity of ash dieback. The location of a tree in one of the ten municipalities of Leipzig was included as random effect.

Based on preceding correlation tests, only uncorrelated numeric variables were chosen. If variables were normally distributed we used the Pearson correlation; otherwise we used the Spearman correlation. If two variables were correlated $\geq |0.65|$, we excluded one of them from subsequent analyses (cf. Dorman and Kühn, 2011).

As there were less trees belonging to one of *F. excelsior*'s varieties than *F. excelsior* itself, we built two models: one model including only individuals of *F. excelsior* from green spaces and streets (model 'site category') and a second model with street trees only, including *F. excelsior* and its varieties *F. excelsior* 'Atlas' and *F. excelsior* 'Westhof's Glorie' (model 'species/variety').

After running a model, model simplification was applied until only significant variables remained (minimal adequate model).

In order to find differences between *F. excelsior* and its varieties, we first ran the minimal adequate model 'species/variety', including *F. excelsior* only. From this model, we randomly drew 999 samples of *F. excelsior* with n = 50 each (according to the number of individuals per variety) from the model's residuals. We then used z-statistic to compare the mean severity of ash dieback of each variety to the distribution of 999 means from the linear mixed effect model. Subsequently, p-values were calculated to determine statistically significant differences. Differences in the severity of ash dieback between varieties were estimated by using a t-test (cf. Dorman and Kühn, 2011).

Results

Level of damage

Symptoms of ash dieback could be observed in all municipalities. 30 % of individual specimens did not show symptoms; thus they were classified as level of damage = 0. The remaining 70 % showed ash dieback of varying severity (Fig. 2, Tab. 1). The proportion of individual trees belonging to level 1 was 20 %. In level 2, proportion was nearly doubled (42.4 %), whereas the proportions of trees from level 3 to 5 remained below 10 %. Only 1 % of trees were severely damaged (level 4) or dead (level 5). Recorded damages like bark beetles and trunk necroses did not show notable results.

Fig. 2

Genetic analyses

In total, 45 samples were tested by applying genetic analyses. Based on DNA fragment length analysis, three of them did not show DNA of *H. fraxineus*, while 42 samples did. These results were verified by additional BLAST-analysis of six samples (Appendix B).

Correlation tests

Before we ran the model, we applied correlation tests. In general, for both model 'site category' and 'species/variety', tree height was correlated with diameter at breast height and year of planting $\geq|0.65|$. Correlation was strongest $\geq|0.80|$ between diameter at breast height and year of planting, and between mean air humidity and mean annual temperature. On account of this we did not include height of trees, diameter at breast height and mean annual temperature into our subsequent analyses.

Model 'site category'

Results of the minimal adequate model were significant for height of crown base (hcrown), year of planting, areas of moderate overheating ('heat2') and feeding marks of bark beetles (Tab. 2). Height of crown base and year of planting showed a negative relationship to level of damage.

Tab. 2

In areas of moderate overheating ('heat2'), trees showed less damage than in cold air areas with good to very good conditions for cold air production ('cold1'). Trees with feeding marks at the trunk were more heavily damaged than trees without feeding marks.

No significance was shown for closeness to water areas, sex, site category, sealing or mean relative air humidity; these variables were excluded during model simplification. And while in green spaces, a higher proportion of trees showed no damages as compared to roadside trees (Tab. 3), there was no significant difference between site categories.

Tab. 3

Model 'species/varieties'

This minimal adequate model was similar to the minimal adequate model 'site category'. Height of crown base, year of planting and feeding marks were significant (Tab. 4). Instead of differences among climate areas, air humidity significantly affected the pathogen. Again, height of crown base and year of planting showed a negative relationship to level of damage, and trees with feeding marks at the trunk were more heavily damaged than trees without feeding marks. Moreover, the level of damage increased with an increase in relative air humidity.
No significance was shown for climate areas, closeness to water areas, sex and sealing, as these variables were excluded during model simplification.

Tab. 4

Differences in ash dieback between species and varieties
Differences in damage by the fungus significantly differed ($p = 0.04$) between variety *F. excelsior* 'Atlas' and *F. excelsior*. There was no significant difference between *F. excelsior* 'Westhof's Glorie' and common ash (Fig. 3). Based on a t-test, there was no significant difference between the two varieties.

Fig. 3

Discussion

The present study elucidates the occurrence, severity and possible causes of *H. fraxineus* in the urban area of Leipzig, Germany, by examining 401 trees. We report the current level of damage by the fungus in Leipzig and discuss parameters influencing it.

Frequency of ash dieback
Symptoms of ash dieback can be found in nearly all municipalities of Leipzig, with 70 % of trees being damaged. It was found that between its first detection in Leipzig's riparian forest in 2011 (Leipzig, 2013) and our survey in 2016, ash dieback had spread all over the city. The existence of *H. fraxineus* at a source right within the city (the riparian forest stretches across Leipzig) has likely promoted the spread of spores by wind (Timmermann et al., 2011) and thus further infections (Metzler, 2011) throughout the city.

Influence of meteorological parameters and combined index of meteorological conditions

Our results show that an increase in mean relative air humidity induces an increase in the level of damage by *H. fraxineus*, verifying Hypothesis a. This is in accordance with Metzler (2011) and Husson et al. (2012), who showed that apothecia of *H. fraxineus* prefer to grow under greater air humidity, leading to an increasing amount of spores and finally to high infection pressure. Still, our model 'site category' showed no effect of mean air humidity. This might be closely related to a higher susceptibility of street trees in model 'species/variety', because local conditions are more stressful (as for this model, only street trees were taken into account). Furthermore, the gradient of mean air humidity across Leipzig (ranging from 75.5 to 77,1 %) might be too small to show an effect. However, air humidity is one meteorological parameter included in the combined index of meteorological conditions, which showed that trees in areas characterised by high temperatures, moderate cooling down at night and reduced relative air humidity ('heat2') are less damaged than those in areas characterised by pronounced cold air and fluctuations in temperature and air humidity ('cold1'; Appendix A; Stadt Leipzig, Amt für Umweltschutz, 2010). Even differences in microclimatic conditions between sites can influence the establishment of fruiting bodies and the developmental stage of the fungus (Cleary et al., 2016). While our measure of air humidity might have been to coarse to show effects, the effect of the combined index of meteorological conditions indicates that climatic drivers of *H. fraxineus* in urban areas are similar to those in non-urban areas (Cleary et al., 2016; Husson et al., 2012; Metzler, 2011). We can therefore also verify Hypothesis c, that occurrence of the fungus (mainly based on observed symptoms) can be associated with the combined index of meteorological conditions, based on climate measurements.

In our study, mean annual temperature and mean air humidity were strongly and negatively correlated, indicating a negative relation of mean annual temperature to level of damage (as opposed to the positive effect of air humidity). Previous studies revealed that the growth of *H. fraxineus* is optimal at 20 °C (Junker, 2013; Kowalski and Bartnik, 2010). If temperature is lower or higher, apothecia grow more slowly, but this reaction can vary among colonies of the fungus (Hauptman et al., 2013; Kowalski and Bartnik, 2010). Similarly, Hauptman et al. (2013) confirmed the negative influence of dry and warm climate on the development of the fungus' fruiting bodies (apothecia), which is in line with our results for Leipzig's combined index of meteorological conditions. However, because we excluded mean annual temperature from our models, we were not able to directly verify Hypothesis b that severity of ash dieback decreases with increasing mean annual temperature.

Influence of sealing

Our assumption that soil sealing under ash crowns reduces damage by *H. fraxineus* could not be verified (Hypothesis d). As nearly 80 % of all trees grew in soil with < 20 % sealing, the lack of obvious differences might result from the lack of variation among sites. It therefore remains to be tested whether the accumulation of leaves on rough surfaces promotes ash dieback, a question that could be addressed in further studies. In addition, we did not find significant differences in the severity of ash dieback between roadside trees and trees in green spaces, so Hypothesis e could not be supported. We assumed that trees in green spaces are more strongly infected by the pathogen. First, because of higher roughness of surrounding area (less soil sealing) and its associated risk of foliage accumulation, representing a reservoir of infectious material (Gross and Holdenrieder, 2013); and second because harsh abiotic conditions along streets make leaves dry off faster (cf. Gross et al., 2014). Regardless of detecting a statistical effect, a higher proportion of street trees were infected by *H. fraxineus*. We suggest that other yet unnoticed effects influence infections, like the distance to the next source of spores or climate conditions of the previous year.

Differences between species and varieties

Differences in damage by the fungus were significant between *F. excelsior* and *F. excelsior* 'Atlas', with 'Atlas' being less damaged. We were therefore able to verify our Hypothesis f that varieties differ in the level of damage by *H. fraxineus*. Previous studies have shown that both common ash and the varieties that we analysed are susceptible to the pathogen (Queloz et al., 2011; Kirisits et al., 2012; Lösing, 2013; Junker, 2013) but to our knowledge, no other studies compared susceptibility of common ash and its varieties. Differences, and also lack of differences, may depend on the combined index of meteorological conditions or on a single meteorological parameter, as their distribution is quite irregular across the city of Leipzig. Consequently, the number of individuals per climate area was unbalanced. In addition, samples of trees were not fully random, as varieties did not occur in all municipalities. As we did not analyze the influence of resistance or genetic differences on the susceptibility to ash dieback, we can neither conclude nor rule out whether these cause the differences that we found.

Conclusion

H. fraxineus is distributed across the whole city area of Leipzig. Differences in its occurrence within the city can be attributed to mean air humidity and most notably to the combined index of meteorological conditions, which combine parameters of temperature and humidity with surface temperature and landscape specific data. Consequently, meteorological differences should be considered when planting ash trees in urban areas. Moreover, the choice of species and their varieties should be considered. Our study includes some uncertainties due to a small range of values of some variables (especially soil sealing), making our data less representative in this respect. We therefore recommend further studies in urban areas to reveal and verify influences of soil sealing on both *H. fraxineus* and ash trees by using modified methods. If soil sealing plays a role in the disease cycle of ash dieback, verifying this would help to improve management of ash dieback.

In general, *H. fraxineus* and its symptoms should be observed on a regular basis in order to record changes in severity, life cycle and occurrence. Future studies should also pay more attention to tree characteristics (like height of crown base, planting year etc.), as there might be a diversity of possible causes for the occurrence of the fungus in urban areas.

Appendix A: Explanation of combined index of meteorological conditions and water areas of Leipzig's map of climate function (modified as per: Stadt Leipzig, Environmental Protection Agency)

Combined meteorological index	
Name	**Description**
Areas of cold air	
cold1	Areas of agriculture: pronounced fluctuations in temperature and air humidity depending on state of vegetation and soil humidity; good to very good production of cold air at night; irritating bioclimate
Summary = Cold air area with good to very good conditions for cold air production	
cold2	Inner-city open spaces (parks, cemeteries, sports facilities, community gardens): depending on the proportion of high vegetation, the daily course of temperature and air humidity as well as the production of cold air at night are more or less pronounced; mitigation of thermal and hygric variability
Summary = Cold air area with moderate to good conditions for cold air production	
cold3	Forest areas: strong modulated daily course of temperature and humidity during growing season; reduction of wind speed; higher volume of cold air but higher temperatures than in cold1; convenient bioclimate
Summary = Cold air area with good to very good conditions for cold air production	
Areas of overheating	
heat1	Climate of city centre: High day and night air temperatures; reduced cooling down at

	night; reduced relative air humidity; strongly reduced air exchange; stressful bioclimate
Summary = Area of intensive overheating	
heat2	Urban climate: Moderately high temperatures; moderate cooling down at night; reduced relative air humidity; restricted air exchange; stressful bioclimate
Summary = Area of moderate overheating	
heat3	Suburban climate: Temperatures lower than in heat1 and heat2; adequate cooling down at night; comparatively good air exchange; convenient bioclimate
Summary = Area of reduced overheating	
Other areas related to climate	
Water area	Large water areas (> 1 ha): reduce variability in temperatures; increase air humidity; favour air exchange

Appendix B: Results of the BLAST-analyses of six DNA samples. The table includes the sample number, fragment length in base pairs (bp), name of the species and results of BLAST-analyses (score, expect, identities, gaps). In case of sample '12151a' the query is shown (retrieved on 2nd May 2018 from: https://blast.ncbi.nlm.nih.gov/Blast.cgi#1090893310).

Sample No.	Fragment	Description	Parameters	
12151a	458 bp	*Hymenoscyphus fraxineus*	Score: Expect: Identities: Gaps:	824 bits (446) 0.0 451/453 (99%) 1/453 (0%)
Query				

```
Score           Expect   Identities       Gaps          Strand
824 bits(446)   0.0      451/453(99%)     1/453(0%)     Plus/Minus

Query  1    TAGAG-CTTAGGGCACCGCCACTGGTTTTAAGGCCCGCCCAAAGGCGCAGCCCAAGACCC  59
            ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  753  TAGAGCCTTAGGGCACCGCCACTGGTTTTAAGGCCCGCCCAAAGGCGCAGCCCAAGACCC  694

Query  60   CGCCGGAGCGGGAGTTGGTCTAAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAAG  119
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  693  CGCCGGAGCGGGAGTTGGTCTAAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAAG  634

Query  120  GGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTA  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  633  GGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTA  574

Query  180  TCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTT  239
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  573  TCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTT  514

Query  240  AACTATTAAATAGTACTCAGACGACACTGTATTTCAAAATTTAGGGTCCTCTAGCAGGCA  299
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  513  AACTATTAAATAGTACTCAGACGACACTGTATTTCAAAATTTAGGGTCCTCTAGCAGGCA  454

Query  300  CAGTCAGCCGAGGCCGACGCCCAGAGGGCGACCTGCTAAAGCAACAATATAATATACACA  359
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  453  CAGTCAGCCGAGGCCGACGCCCAGAGGGCGACCTGCTAAAGCAACAATATAATATACACA  394

Query  360  AGGGTGGGGTTTCTACCCGTGAGGGCAGGAACTCTGTAATGATCCTTCCGCAGGTTCACC  419
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  393  AGGGTGGGGTTTCTACCCGTGAGGGCAGGAACTCTGTAATGATCCTTCCGCAGGTTCACC  334

Query  420  TACGGAACGCGCTAGCAGTCAGGTTCCCCCAGC  452
            ||||||||||||||||||||||||| |||||||
Sbjct  333  TACGGAACGCGCTAGCAGTCAGGTTTCCCCAGC  301
```

12151b	459 bp	*Hymenoscyphus fraxineus*	Score: Expect: Identities: Gaps:	830 bits (449) 0.0 452/453 (99%) 1/453 (0%)
12156b	390 bp	*Hymenoscyphus fraxineus*	Score: Expect: Identities: Gaps:	717 bits (388) 0.0 388/388 (100%) 0/388 (0%)
12164a	462 bp	*Hymenoscyphus fraxineus*	Score: Expect: Identities: Gaps:	833 bits (451) 0.0 454/455 (99%) 1/455 (0%)
12180a	460 bp	*Hymenoscyphus fraxineus*	Score: Expect: Identities: Gaps:	839 bits (454) 0.0 456/457 (99%) 0/457 (0%)
12181a	455 bp	*Hymenoscyphus fraxineus*	Score: Expect: Identities: Gaps:	826 bits (447) 0.0 452/454 (99%) 0/454 (0%)

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Fig. 1: Tree with typical symptoms of ash dieback (left) at the Louise-Otto-Peters Allee in the city of Leipzig.

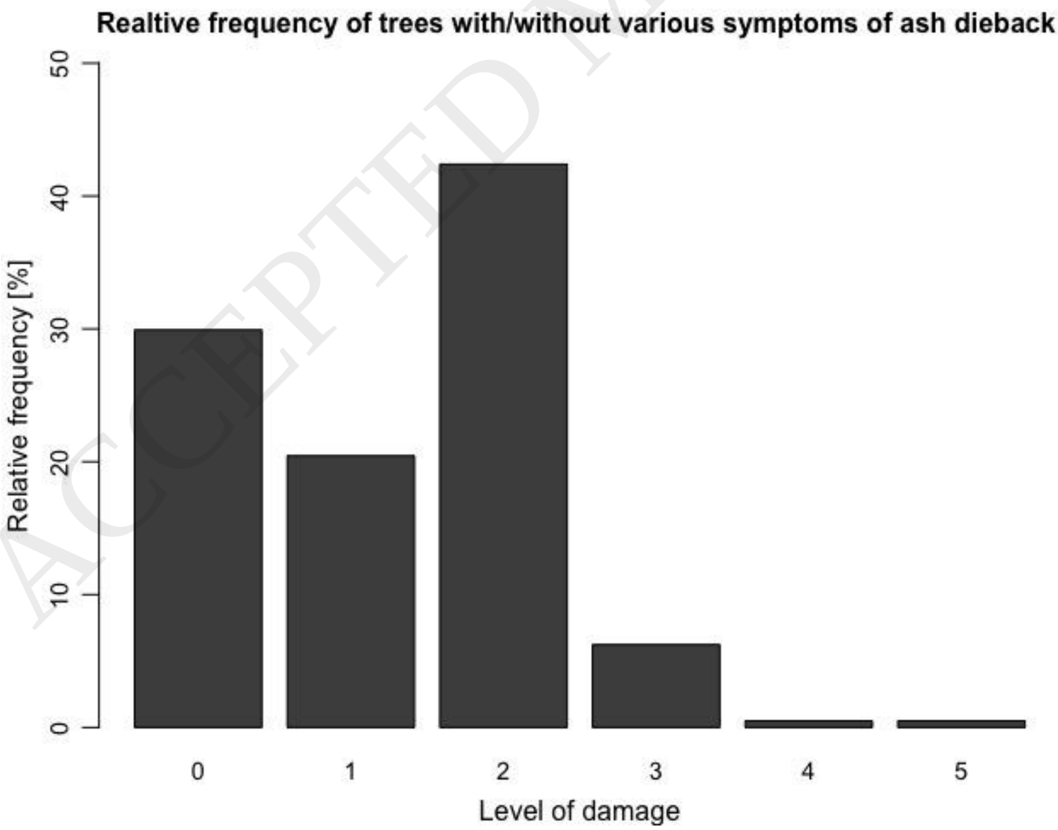

Fig. 2: Relative Frequency of damage level of ash dieback (total data set; n = 401).

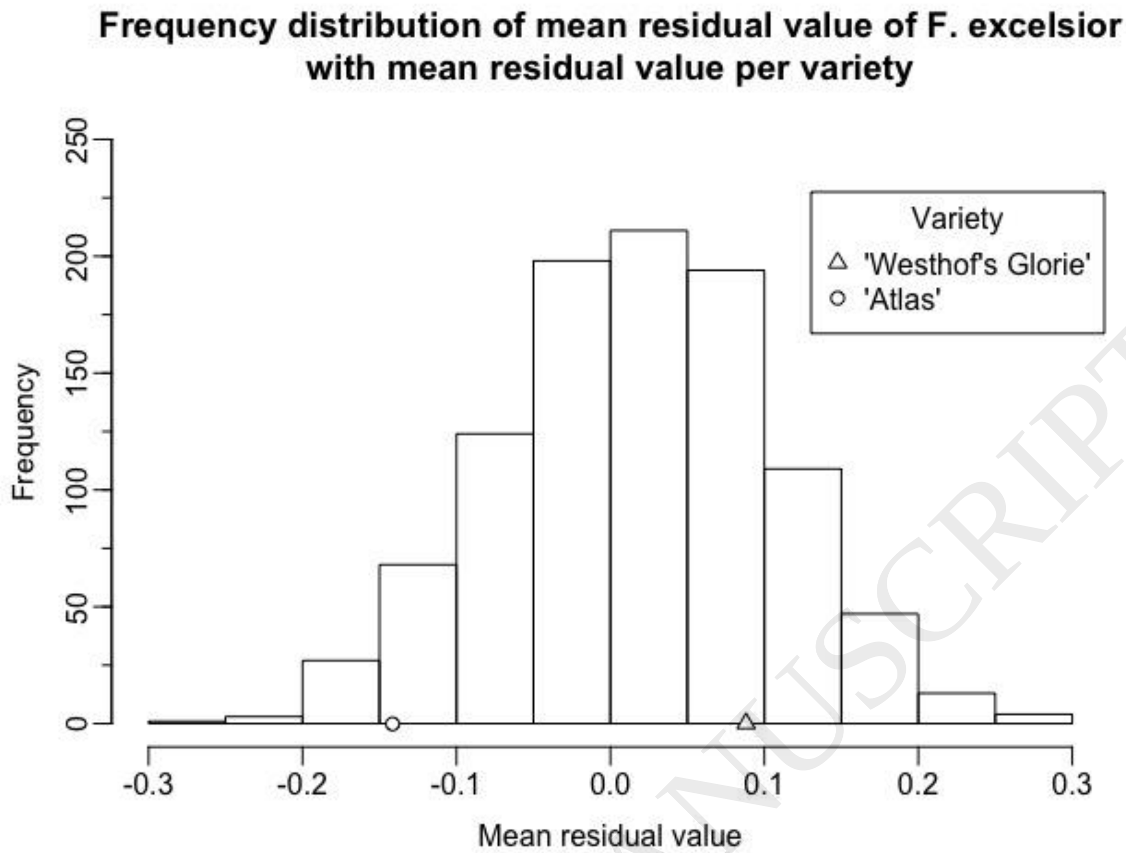

Fig. 3: Frequency distribution of 999 mean residual values of model 'species/varieties' based on *F. excelsior* (frequency histogram) according to mean residual values of varieties (circle = 'Atlas'; triangle = 'Westhof's Glorie'), indicating differences in the level of damage by ash dieback.

Figure legend

Tables

Tab. 1: List of damage levels of ash dieback with declaration to crown condition and further tree characteristics (extracted from: NW-FVA Abt. Waldschutz, 2013), as well as levels of leaf loss (extracted from: Lenz et al., 2012)

Level of damage	Description of damage levels	Leaf loss in percent
0	- Vital ash - Crown without symptoms of ash dieback - No loss of foliage - No bark beetle infestation	0 to 10
1	- Reduced foliage - No shoot damages typical of ash dieback - No bark beetle infestation at stem zone	11 to 30
2	- Thinned foliage and first symptoms of ash dieback in margin areas of the crown: brownish young shoot apexes - No bark beetle infestation - If pressure of infestation is high, secondary pathogens start to establish (i.e. visible rhizomorphs of *Armillaria* sp. or *Flammulina* sp.)	31 to 50
3	- Increasingly thinned crown - Large amount of dead wood and typical symptoms of ash dieback: brownish young shoot apexes - Scattered bore holes of bark beetles - If pressure of infestation is high, secondary pathogens start to establish (i.e. visible rhizomorphs of *Armillaria* sp. or *Flammulina* sp.)	51 to 80
4	- Desiccation from outer to inner crown; inner crown with clusters of foliage - Large amount of deadwood and typical symptoms of ash dieback - Partially dry branches - Bark beetles start to occupy the tree (i.e. *Hylesinus fraxini* Panzer (1779) and *H. crenatus* Fabricius (1787) - Visible infestations with secondary pathogens (i.e. *Armillaria* sp.)	81 to 99
5	- Tree is dead or dies - No foliage; partially epicormic shoots at stem zone - Intense bark beetle infestation in the crown and at the stem	100

	- Decay of wood at stem base and root zone - Partially necrotic lesions at stem base	

Tab. 2: Minimal adequate model of model 'site category', showing tree characteristics and abiotic parameters affecting *H. fraxineus* in the city of Leipzig, Germany: Structure of Model with random and fixed effects; significant results and associated categories; Value, standard deviation (Std.Error), degree of freedom (DF), t-value and p-value. hcrown = height of crown base; for climate areas, see Tab. 1.

Random effects:

Formula: ~1 | municipality

	(Intercept)	Residual
StdDev:	0.25793	0.9365897

Fixed effects: damage ~ hcrown + year of planting + climate area + feeding marks

	Value	Std.Error	DF	t-value	p-value
(Intercept)	13.25	4.88	278	2.72	< 0,05
hcrown	-0.09	0.03	278	-2.88	< 0,05
year of planting	-0.01	0.00	278	-2.37	< 0,05
climate area cold2	-0.20	0.17	278	-1.16	> 0,05
climate area cold3	-0.23	0.23	278	-0.98	> 0,05
climate area heat1	-0.31	0.20	278	-1.54	> 0,05
climate area heat2	-0.51	0.18	278	-2.80	< 0,05
climate area heat3	0.20	0.38	278	0.53	> 0,05
feeding marks	0.31	0.14	278	2.24	< 0,05

Tab. 3: Survey of site categories and their proportion per level of damage (absolute and relative), sum of individuals per level of damage and per category.

	Level of damage (absolute and relative)												**Sum of individuals and percent per site category**	
Site category	0	%	1	%	2	%	3	%	4	%	5	%		
Green space	55	36,7	22	14,7	62	41,3	10	6,7	-	0,0	1	0,6	150	100
Street	30	20,5	33	22,6	72	49,3	10	6,8	1	0,7	-	0,0	146	100
Sum of individuals per level and mean %	85	28,7	55	18,6	134	45,3	20	6,8	1	0,3	1	0,3	296	100

Tab. 4: Minimal adequate model of model 'species/varieties', showing tree characteristics and abiotic parameters affecting *H. fraxineus* in the city of Leipzig, Germany: Structure of model with random and fixed effects; significant results and associated categories; Value, standard deviation (Std.Error), degree of freedom (DF), t-value and p-value. hcrown = height of crown base.

Random effects:

Formula: ~1 | municipality

	(Intercept)	Residual
StdDev:	0.3435234	0.8896475

Fixed effects: damage ~ hcrown + year of planting + feeding marks + air humidity

	Value	Std.Error	DF	t-value	p-value
(Intercept)	-34.15	22.75	237	-1.50	> 0,05
hcrown	-0.22	0.06	237	-3.91	< 0,05
year of planting	-0.01	0.00	237	-2.21	< 0,05
feeding marks	0.35	0.13	237	2.65	< 0,05
air humidity	0.67	0.29	237	2.35	< 0,05