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WP3 Practical solutions for protection, management and eradication

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Report on ash tree varieties less susceptible or resistant to *Chalara fraxinea* and their availability to practice

DOCUMENT INFORMATION

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2. Revision History

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3. Dissemination Level

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EXECUTIVE SUMMARY

In many parts of Europe the ash (*Fraxinus excelsior* L.) is an important native species in forestry and landscaping. Moreover, common ash and its many cultivars together with other ash species are important in urban green infrastructure all over Europe. However, in large parts of Europe ash trees are dying from Ash dieback disease caused by a newly introduced fungal pathogen, *Hymenoscyphus fraxineus*, previously called *Chalara fraxinea or Hymenoscyphus pseudoalbidus.* Currently, no effective measures to fight the disease in forests and urban plantings are available, and losses caused by the disease are serious. However, early studies in several European countries have shown that certain individuals of *Fraxinus excelsior* may have increased levels of genetic resistance and that species and cultivars may differ in their level of susceptibility. So far resistance testing by different groups in Europe has been directed almost exclusively towards germplasm for forestry purposes whereas the *Fraxinus* species are important trees in urban forestry as well.

Therefore within the EMPHASIS project testing for resistance against *Hymenoscyphus fraxineus* in the variety of ash species and cultivars for urban plantings available in tree nursery industry was one of the tasks in Work Package 3. For that purpose large scale screening experiments with a range of ash species and cultivars were carried out in The Netherlands and in the Czech Republic. Additionally surveys of the ashes in urban areas in Latvia and in the Czech Republic were carried out to identify remaining healthy ash trees (Latvia) and to investigate differences in disease levels between common species and cultivars in practice (Czech Republic).

The results of the resistance testing confirm that species as well as cultivars within a species clearly differ in susceptibility to the disease. In a field experiment in The Netherlands in the period 2016-2018, 15 *Fraxinus* species and 25 cultivars were tested by means of artificial infection with *Hymenoscyphus fraxineus.* A small piece of wood infested by this fungus was inserted under the bark on the stems of young trees and resulting symptoms (stem lesions, internal discolouration, dieback of shoots) were recorded. From the species tested, *F. excelsior* and *F. angustifolia* appeared to be very susceptible, whereas *F. americana*, *F. ornus*, and *F. pennsylvanica* developed no or only limited symptoms. Of the less common species *F. manchurica* and *F. spaethiana* tested very resistant, *F. biltmoreana*, *F. latifolia*, *F. pallisae* and *F. velutina* developed only (very) limited symptoms, whereas *F. xanthoxyloides var. dumosa* and a *F. chinensis* cultivar (‘Emma’s Gold’) appeared very susceptible. Within the susceptible *F. excelsior* the cultivars tested clearly differed in susceptibility to ash dieback with especially ‘Diversifolia’, ‘Eureka’, ‘Geessink’ and ‘Allgold’ performing much better than most other cultivars. In a set of similar experiments in the Czech Republik with six *Fraxinus* species, *Fraxinus chinensis*, has been found to have significant resistance. The most damaged species were European ash *Fraxinus excelsior* and Asian ash *Fraxinus sogdiana* whereas in *F. pennsylvanica* and *F. ornus*, also a higher level of resistance was found compared to *F. excelsior*.

The results of the survey of ash trees in urban areas are very much in line with these test results. The examination of 1.209 individuals of five species and six cultivars in two cities in the Czech Republic revealed significant differences in infection percentages between species as well as between cultivars of *F. excelsior.* The *Fraxinus* species most damaged was *F. excelsior* and most of its cultivars have been badly affected too with especially *F. excelsior* 'Nana' being the exception with results comparable to the much less affected *F. pennsylvanica* and *F. americana*. The species least affected in this survey was *F. ornus*. Additionally a survey of over 300 ash trees in 16 Latvian cities and villages revealed that about 15% of the ash trees was still without symptoms of Ash dieback disease. During the survey many cases of insect attack, especially by bark beetles, and an imbalance in nutrients were recorded. Based on the results it was concluded that limited availability of nutrients, especially Sulphur, and insect damage may have contributed to weakening the trees which making them more susceptible to other stresses such as Ash dieback disease.

1. INTRODUCTION

The ash (*Fraxinus excelsior* L.) is an important native species in forestry and landscaping in many European countries. In addition common ash and its many cultivars together with other ash species (including *F. americana*, *F. nigra*, *F. ornus*, *F. pennsylvanica* and *F. velutina*) are important in urban green infrastructure all over Europe. However, in large parts of Europe ash trees are dying from *Hymenoscyphus fraxineus*, previously called *Chalara fraxinea or Hymenoscyphus pseudoalbidus*, a newly introduced fungal pathogen causing Ash dieback disease also known as Chalara disease. First observed in eastern Europe in the mid-1990s (see Kowalski and Holdenrieder, 2009), the disease has spread over northern and central European countries up to, recently, the UK (Forestry Commission, 2013). The effects of the disease are dramatic. In Lithuania for example the disease caused a decrease of the ash forest area from 53,000 to 38,000 hectares between 2001 and 2009 (European Commission DG Environment News Alert Service Issue 313, 17 December 2013) and in Denmark no ash trees at all are being planted after the impact of the disease became apparent. In a very recent study on mortality rates from ash dieback in Europe mortality up to 70% in natural woodlands and up to 85 % in ash plantations is reported (Coker *et al.* 2019).

Currently, no effective measures to fight the disease in forests and urban plantings are available. However, early studies in Sweden (Stener; 2012), Denmark (McKinney *et al*.; 2011; Nielsen *et al*., 2012), Lithuania (Pliura *et al*.; 2011) and the Netherlands (Kopinga & de Vries, 2012) have shown that certain individuals of *Fraxinus excelsior* may have increased levels of genetic resistance. On the long run this will provide a way to deal with this disease, especially in forestry, by enabling the breeding and selection of more resistant germplasm of *Fraxinus excelsior*.
In the urban environment a faster solution is needed as *Fraxinus* species and cultivars are important elements of the green infrastructure that cannot easily be missed, especially with other important urban tree species being threatened by serious diseases and pests as well (e.g. Massaria in *Platanus*, Chestnut disease in *Aesculus*, Oak processionary moth in *Quercus* species, etc.) and several other invasive pests and diseases being ready to enter Europe (Tomiczek, 2014). Preliminary research demonstrated clear differences in susceptibility in the many different ash cultivars and species that are being used in urban green and with some species seemingly not being affected by Ash dieback disease (Lösing, 2012).
Therefore within the EMPHASIS project testing for resistance against *Chalara fraxinea* in the variety of ash species and cultivars for urban plantings available in tree nursery industry was included as one of the tasks in Work Package 3. Additionally in 16 cities and villages in Latvia as well as in 2 cities in the Czech Republican inventory of *Fraxinus excelsior* in the urban areas has been done to identify individuals more resistant to infection by *Hymenoscyphus fraxineus* (*Chalara fraxinea*).

Thus the research on ash dieback disease within the Emphasis project focused completely on evaluation of susceptibility levels in existing species and cultivars in an effort to provide urban green managers with information needed for efficient management of the ash dieback problem in urban forestry. The results on the one hand should provide information on disease risk in the existing urban ash plantings and on the other hand should provide urban green managers with alternatives for the ash species and cultivars that are threatened most seriously by Ash dieback. The research did not include investigations into the genetic background of resistance to the disease, but the results provide a starting point for work in this area done elsewhere as it provides information about susceptibility levels in a vast array of *Fraxinus* species and cultivars.

2. SCREENING FOR RESISTANCE

Introduction

Young trees of different *Fraxinus* species and cultivars were brought together in test plots in two different countries (CZ and NL) and tested for resistance by means of artificial infection with *Hymenoscyphus fraxineus*. In this experiments the resulting external lesions on the stems and internal discoloration were used as a measure for the level of susceptibility of individual species or cultivars. Building on preliminary results in literature and through a close co-operation with participants from the tree nursery sector it was possible to complete this work within the project period of 4 years and to come up with guidelines for managers of urban green which species and cultivars can be used to reduce future losses by ash dieback disease and to maintain the important role of *Fraxinus* species and cultivars in urban green infrastructure.

Screening of *Fraxinus* species and cultivars in the Netherlands

In a large field experiment a large collection of *Fraxinus* genotypes was tested for resistance to ash dieback. In 2015 much effort was done to collect all ash species and cultivars available in the Dutch tree nursery industry as well as more rare introductions from arboreta and private collections. From all genotypes propagation material was collected and grafted onto seedling rootstocks. This resulted in 1114 young trees of 15 species and 25 cultivars that were planted on an experimental field early in 2016 (Figure 1). In 2016 and during the first part of the growing season of 2017 the trees were left to grow until the inoculation in August 2017. Due to several causes including graft incompatibility, bad regrowth and feeding damage by animals during this period. In 2007 about 750 trees were selected to be inoculated to test their resistance/susceptibility to infection by *Hymenoscyphus fraxineus.*

Figure 1. Experimental field with collection of ash species and cultivars to be tested for resistance to ash dieback caused by Hymenoscyphus fraxineus; at the time of planting in April 2015 (left) and the same field at the time of inoculation in August 2017 (right).

In August 2007 the trees were inoculated by placing a small wood chip colonized by the pathogen between the bark and the wood on the stem of the trees (Figure 2). Two different isolates, one from the Netherlands and one from Czech Republic, were used because of the variability within the pathogen. In the following growing season the plants were examined for external symptom development (wound not closing, lesion development, top dieback) in July. Finally in August stem samples were collected of all inoculated trees and transported to the laboratory where the length of external lesions and the length and percentage of internal discoloration were measured (Figure 3).

Figure 2. Inoculation procedure; fungal colony on agar plate with small wood chips (left) that are inserted into the stems by making a cut (center left) placing the wood chip in the wound (center right) and closing the wound by wrapping with parafilm (right).

Figure 3. Collection of stem samples of inoculated trees (left) and processing of the samples in the laboratory (right).

Symptom development in the field was rather limited, in many cases the wounds had closed completely (DI 0) or partially (DI 1a and 1b) but without external symptoms developing. However, in some species and cultivars small or large lesions on the stem (DI 2 and 3), top dieback (DI 4) and in a rare case even death of a tree (DI 5) were observed (Figure 4). Nevertheless the results of the symptom assessment in the field showed clear differences between species and within species between cultivars (Figure 5). Similarly the rating of the internal discolorations, if present, showed clear differences between genotypes (Figure 6).

Figure 4. Inoculated tree without symptoms, wound closed, no lesion & shoot alive (left) and tree with lesion around the inoculation wound, often shoot dead (right).

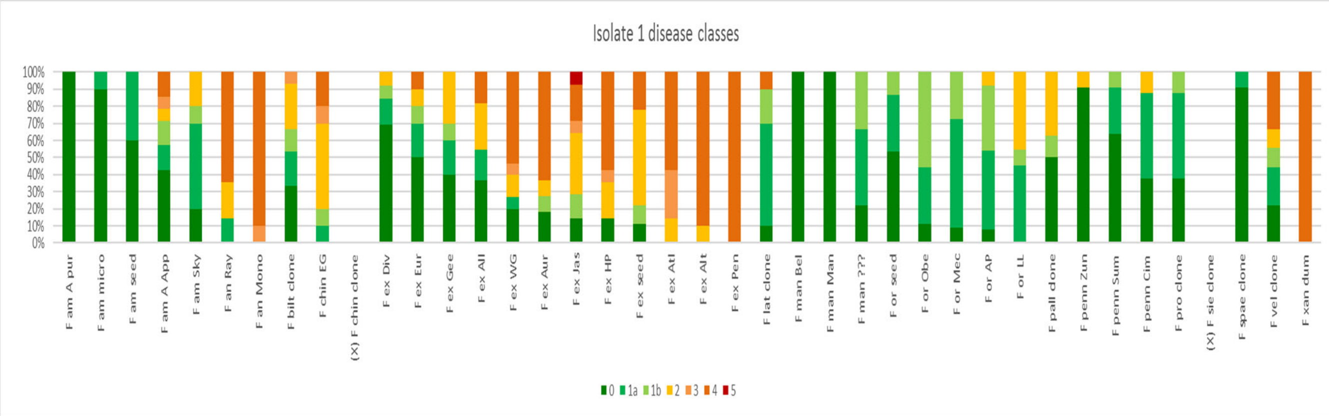

Figure 5. External symptoms of Fraxinus species and cultivars after stem-inoculation with Hymenoscyphus fraxineus (NL-isolate) for all genotypes that were tested.

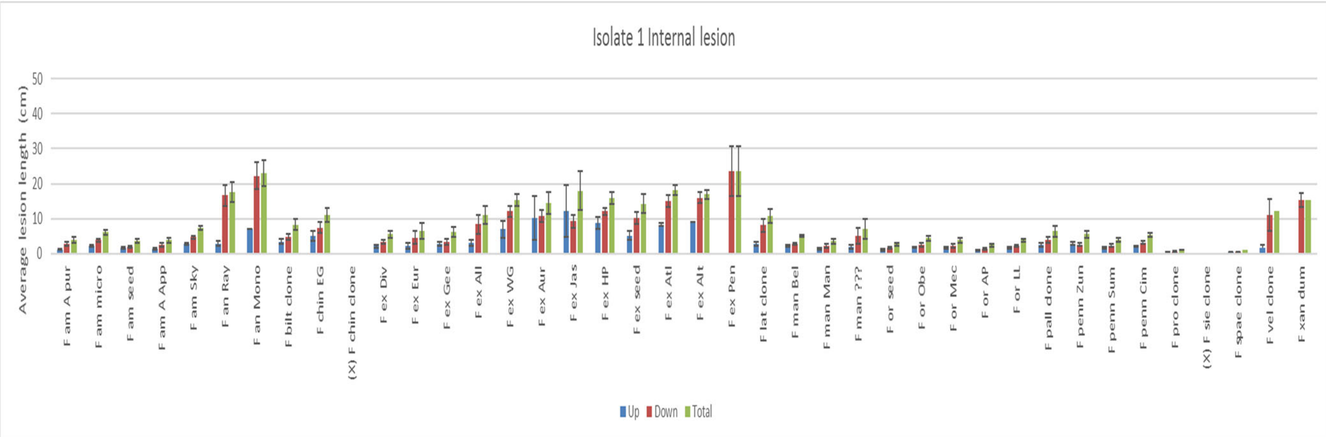

Figure 6. Internal symptoms of Fraxinus species and cultivars after stem-inoculation with Hymenoscyphus fraxineus (NL-isolate) for all genotypes that were tested; upward, downward and total length of the discoloration in the stem xylem.

Differences between species and cultivars as revealed by the different criteria were very consistent. On a species level especially *F. excelsior* and *F. angustifolia* appeared to be very susceptible, whereas *F. americana*, *F. ornus*, and *F. pennsylvanica* developed no or only very limited symptoms. Of the less common species *F. manchurica* and *F. spaethiana* tested very resistant, *F. biltmoreana*, *F. latifolia*, *F. pallisae* and *F. velutina* developed only (very) limited symptoms, whereas *F. xanthoxyloides var. dumosa* and a *F. chinensis* cultivar ('Emma's Gold') appeared very susceptible. Within the susceptible *F. excelsior* the cultivars tested clearly differed in susceptibility to ash dieback with especially 'Diversifolia', 'Eureka', 'Geessink' and 'Allgold' performing much better than most other cultivars.

Screening of *Fraxinus* species in Czech Republik

Several infection trials for testing the level of resistance of European and non-European *Fraxinus* species to ash dieback were carried out. Treatments have been realized on 400 individuals of six European, American and Asian species of ashes (*F. excelsior*, *F. ornus*, *F. americana*, *F. pennsylvanica*, *F. chinensis* and *F. sogdiana*). In the inoculation trials eight strains of *H. fraxineus* were used (Table 1). This number of cultures was selected for the elimination of huge differences in aggressiveness and virulence between strains of this pathogen. Inoculation was carried out using two methods. The first was to use a part of a culture of the pathogenic fungus injected directly into the host tissue (under bark). The second way respects the natural way of infection. In that case a part of a culture was attached to a leaf scar freshly breakaway sheet according to Kräutler *et al*. 2015.

Table 1. List of cultures of *H. fraxineus* with dates and locations of collecting and mycelia characteristics

Cultures	Year of collection	Locality	Mycelial characteristics	
1714	2013	Czech republic_Highlands	Fast growing	light
1731	2013	Czech republic_Lužické hory	Fast growing	light
B/1/19	2009	Austria_Bisamberg	Fast growing	light
VER/2	2009	Austria_Verditz, Carinthia	Fast growing	light
BIZ/1	2010	Austria_Bizau, Vorarlberg	Slowly growing	dark
NOR 1/23	2010	Norway_Oslo	Slowly growing	dark
1732	2013	Czech republic_Lužické hory	Slowly growing	dark
1653	2013	Czech republic_Brno	Slowly growing	dark

Statistical analyses confirmed significant differences between tested ash species. *Fraxinus chinensis*, which has been found to have significant resistance to both external (Figure 7) and internal infections (Figure 8), was the best performing species. The most damaged species are European ash *Fraxinus excelsior* and Asian ash *Fraxinus sogdiana*. In the other species tested, *F. pennsylvanica* and *F. ornus*, also a higher level of resistance was found compared to *F. excelsior*.

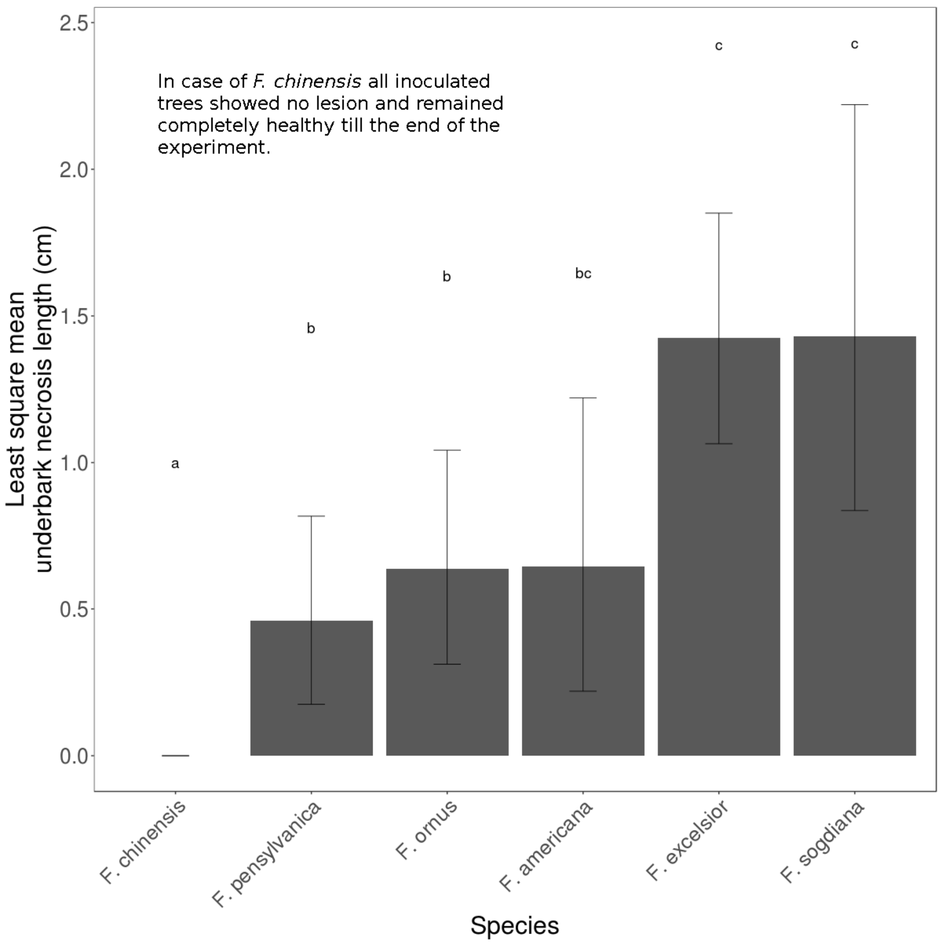

Figure 7. Results of resistance testing by means of artificial infection on stems of 6 Fraxinus species measured by the size of the resulting bark lesions.

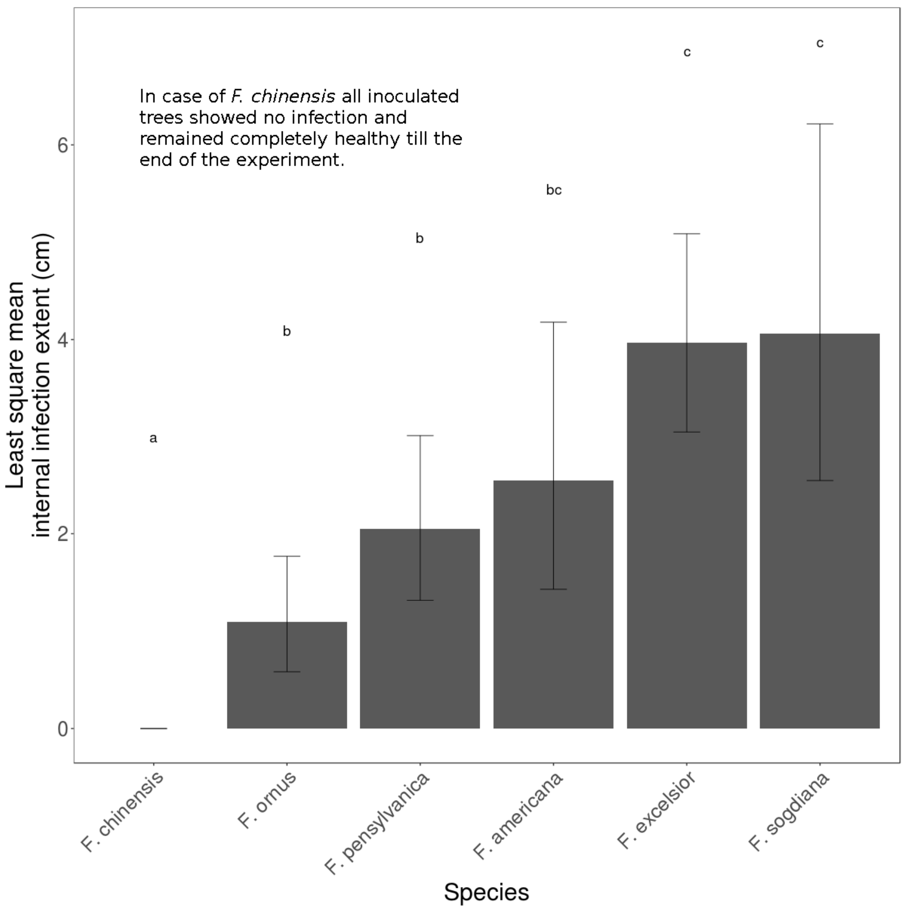

Figure 8. Results of resistance testing by means of artificial infection on stems of 6 Fraxinus species measured by the length of the resulting internal discolorations.

3. SURVEY OF ASH TREES IN URBAN AREAS

Survey of urban ash trees in Czech Republic

In collaboration with the Public Green Areas in Brno four urban research plots with occurrence of pathogenic fungus *Hymenoscyphus fraxineus* were chosen (N 49°12.44677', E 16°36.36035'). The research was launched on these areas in 2016. With the city Kroměříž it was agreed to establish two more research areas in the urban environment (N 49°17.90255', E 17°23. 58343').

Until now 1.209 individuals of five species and six cultivars have been examined for symptoms of ash dieback. Significant differences were observed in the infection percentages between species as well as between cultivars of *F. excelsior* (e.g. cv. Nana vs cv. Pendula) (Figure 9). Selected cultivars and species will be tested for resistance in situ in the future with the aim of possible application as resistant replacement for seriously affected cultivars. The *Fraxinus* species most damaged is *F. excelsior* and most of its cultivars have been badly affected too. The exception is *F. excelsior* 'Nana', the results of which were very good, comparable to those of American *Fraxinus* species (*F. pennsylvanica* and *F. americana*). The species least affected in this survey was *F. ornus*.

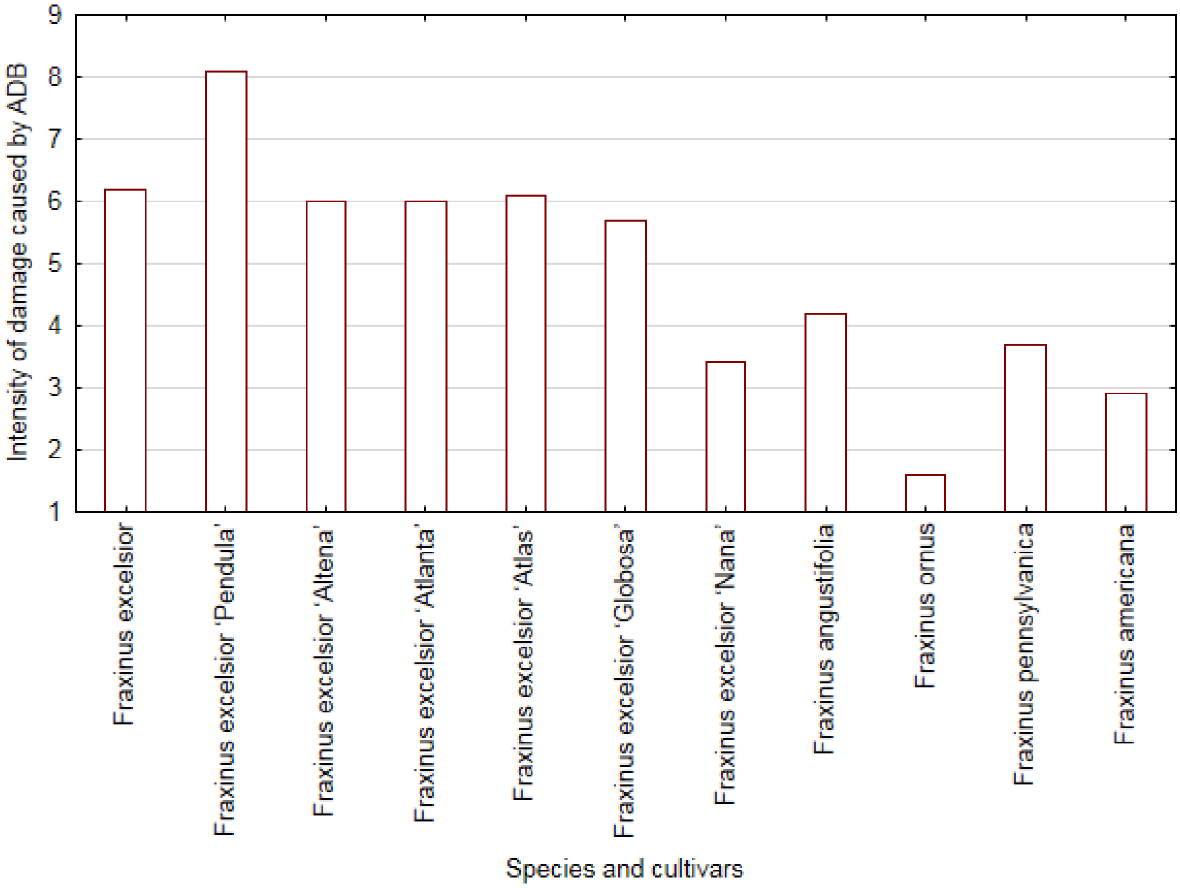

Figure 9 Intensity of ash dieback in ash tree species surveyed in the urban areas of the cities Brno and Kroměříž (Czech Republik)

Survey of urban ash trees in Latvia

A survey of *F. excelsior* trees in the urban areas of Latvia has been carried out by IAS in the years 2015 - 2018 (Table 2) to identify healthy individuals more resistant to infection by *H. fraxineus* (Figure 10).

Table 2. Results of the inventory / survey of ash trees in Latvia, year 2015-2018.

№	City / Village / Region Location coordinates	Description of ash trees	Confirmed presence of *Chalara fraxinea*	Insects recorded
1	Strenči N 57.628226, E 25.689646	6 separate , healthy ash trees	6 No	*Hylesinus fraxini*
2	Murjāņi N 57.134819, E 24.666041	7 Planted trees in the alley - ash simultaneously with linden alley	7 Yes	*Hylesinus oleiperda*
3	Valmiera N 57.538675, E 25.423197	2 healthy ash tree	2 No	*Hylesinus fraxini*
4	Kocēni N 57.522144, E 25.336000	12 ash trees - old ash trees avenue	12 Yes	*Hylesinus fraxini*
5	Straupe N 57.355633, E 24.952016	2 separate, healthy and 4 invaded ash trees in the urban environment	2 No / 4 Yes	*Hylesinus fraxini* *Hylesinus oleiperda*
6	Rubene N 57.467584, E 25.256158	5 young ash trees	5 Yes	*Hylesinus fraxini* *Hylesinus oleiperda*
7	Jaunjelgava N 56.614040, E 25.082007	4 healthy ash trees	4 No	*Hylesinus fraxini*
8	Sigulda N 57.165535, E 24.85140	238 ash trees	15 No / 223 Yes	*Hylesinus fraxini* *Hylesinus oleiperda*
9	Varaklāni N 56.606348, E 26.777425	2 healthy ash trees	2 No	*Hylesinus fraxini*
10	Rīga N 56.952605, E 24.104726	2 healthy ash trees	2 No	-
11	Lestene N 56.777425, E 23.146435	2 healthy ash trees	2 No	-
12	Auce N 56.468843, E 22.889957	3 old ash trees	3 Yes	*Hylesinus fraxini*
13	Dobele N 56.623167, E 23.272389	5 ash tree (Ø >60 cm)	1 No / 4 Yes	*Hylesinus fraxini* *Hylesinus crenatus*
14	Mālpils N 57.014399, E 24.889344	7 (5..6 years old) and 15 (1..3 years old) ash trees	16 No / 6 Yes	*Hylesinus fraxini*
15	Allaži N 57.026243, E 24.813352	17 ash tree (Ø >40 cm)	17 Yes	*Hylesinus fraxini* *Hylesinus crenatus*
16	Kabile N56.951987, E 22.401125	6 ash tree (Ø >40 cm)	6 Yes	*Hylesinus fraxini* *Hylesinus crenatus*
	Total	**339 ash trees**	**52 No /287 Yes**	

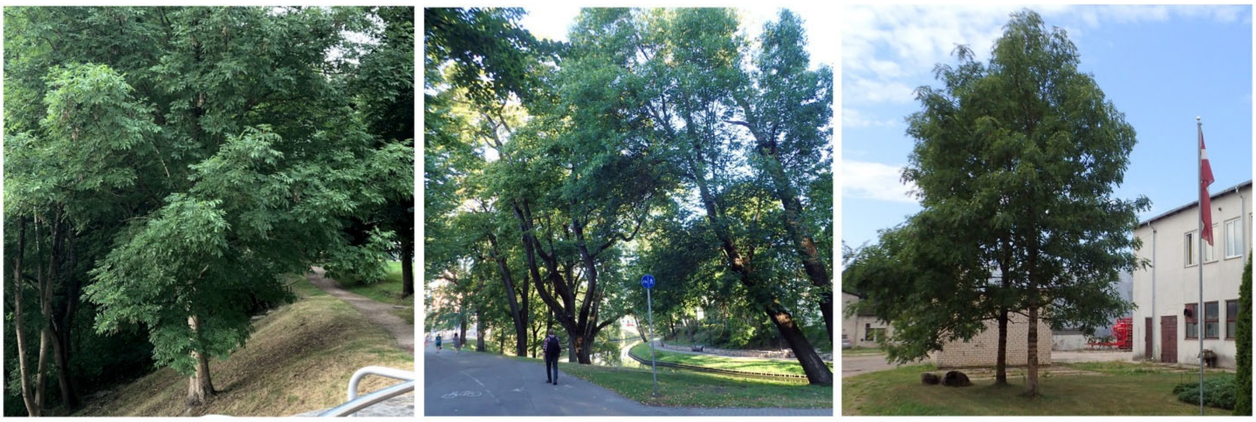

Figure 10. Three survey places of ash trees in Latvia, year 2015-2018.

The inventory / survey of ash trees by Integrētās Audzēšanas Skola (IAS) in urban environment of Latvia 2015-2018 confirms evidence of the insect–plant diseases relationships. *Hymenoscyphus fraxineus (Chalara fraxinea)* infection on weakened trees was caused by lack of nutrition, soil conditions or insect damage. Weakened trees to the point where it succumbs are more readily attacked by other pests or pathogens (UK Forestry Commission, 2016).

Adult/larva damage of *Leperisinus varius (Hylesinus fraxini)* ash bark beetle (a); *Hylesinus toranio (Hylesinus oleiperda)* bark beetle (b), *Hylesinus crenatus* great black ash bark beetle (c) was found on healthy trees and trees dying with ash dieback. This insect-disease combination was found on young, old and very healthy ash trees (Malpils, Sigulda, Straupe). Bark beetles (family *Scolytidae)* are vectors of fungal pathogens in trees. By digging galleries in bark and phloem, bark beetle by body covered with fungi spores disperse disease.

Nutrition, soil and environmental conditions are primary, pests secondary (nutrition-insect–plant diseases relationships). There is a significant difference of these interactions in the EU countries with different climate zone. Combination of all or some of these factors might contribute to the death of the trees, making it difficult to determine the main cause of death.

Importance of nutrition for vitality and resistance of ash.

Limitations of nutrients can reduce growth and increase the susceptibility of ash to other stress factors. Individual trees differ in their demand for nutrients, but require a similar optimal balance.

An important factor influencing the vitality of trees is eutrophication of the soil (environment), when the nitrogen and sulfur deposit changes the macro-and micro elemental ratio in the soil, disrupting the ash tree feeding regime and affecting the physiological processes of the trees (Kenigsvalde et al., 2010). Because of the close relationship between S and N, metabolism of N will be upset by S deficiency. Sulfur is required by trees for synthesis of three amino acids – cystine, cysteine and methionine -, which are essential components of protein for formation of chlorophyll. Deficiencies of sulfur cause serious plant health problems and loss of vitality.

Sulphur deficiency (Figure 12) is the most widespread current nutrient disorder in crops in Europe and in some other parts of the world. This follows environmental regulations limiting SO2 emission on aerial and industrial pollution (atmospheric sulphur deposition has decreased drastically; Figure 11) and the use of sulphur-free urea and superphosphate fertilizers (Schnug & Haneklaus, 1998). Between 1970 and 2016, emissions of acidifying gases in the European Union (EU) went down significantly. The sulphur oxide emissions (SO2) decreased by almost 90%.

Emissions of acidifying gas SO_2

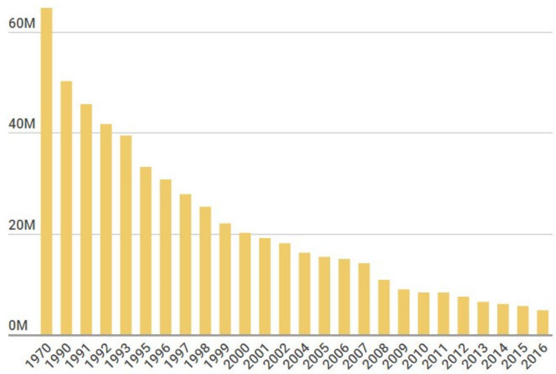

Figure 11. Emissions of acidifying gas SO_2 (in million tonnes), Eurostat.

Sulphur deficiency on top of ash

Figure 12. Sulphur deficiency on top of ash tree in July, Latvia

The key role of Sulphur in plant disease for many plants becomes increasingly apparent. S is highly toxic (ED50 1–3µg ml-1) to many fungal pathogens representing *ascomycetes, basidiomycetes, and deuteromycetes* (Cooper, 2004). The plant deficit of Sulphur is favourable now for many plant diseases in agricultural and forestry crops (Table 3).

Table 3. Pathogens that may be affected by Sulfur levels

Ascomycetes:	***Basidiomycetes:***
Hymenoscyphus f. (Chalara fraxinea) – ash dieback	*Armillaria spp.*- root rot diseases
Ceratocystis ips - blue stain fungus	*Heterobasidion spp*. - root rot of conifers
Ophiostoma ulmi - Dutch elm disease	rusts
Microsphaera alphitoides - powdery mildew of oak	and other diseases.
Uncinula bicornis - powdery mildew of maple	
and many others diseases	

Would be useful to establish locally by EU countries the IPM for more resistant ash trees in urban environment with nutrition-insect-plant diseases relationship in climate-soil specific conditions. With correct measures taken that, would increase understanding of ash resistance to dieback in urban environment and for forestry sector to save the native ash trees for future.

4. CONCLUSION

The work reported in this deliverable was aimed assessing the levels of susceptibility to Ash dieback disease caused by *Hymenosyphus fraxineus* in a large collection of ash species and cultivars in order to provide urban green managersand the tree nursery sector with information on disease risk in existing urban ash plantings on the one hand and on alternatives for the ash species and cultivars that are threatened most seriously by Ash dieback on the other hand. For this purpose large scale screening experiments including many different ash genotypes (species as well as cultivars) were performed in The Netherlands and in Czech Republic. Additionally surveys investigating differences in disease levels ash trees affected by Ash dieback in urban areas were performed in Latvia and Czech Republic.

The results of the screening experiments in The Netherlands and in Czech Republic were very much in line with each other. The method used, stem-inoculation of young trees by means of inserting a small piece of infected ash wood under the bark on the stem, resulted in successful infections and the variation in symptom development enabled characterization of the levels of susceptibility of the tested species and cultivars. The results confirm that within the genus *Fraxinus* the species, and within species cultivars, vary in susceptibility to Ash dieback. Also differences in susceptibility as revealed by the different criteria (outer symptoms, internal discoloration) were very consistent.
On a species level especially *F. excelsior*, *F. sogdiana* and *F. angustifolia* appeared to be very susceptible after inoculation, whereas *F. americana*, *F. ornus*, and *F. pennsylvanica* developed no or only limited symptoms. Of the less common species *F. manchurica* and *F. spaethiana* tested very resistant, *F. biltmoreana*, *F. latifolia*, *F. pallisae* and *F. velutina* developed only limited symptoms, whereas *F. xanthoxyloides var. dumosa* appeared very susceptible. The results for *F. chinensis* were not very conclusive; in the Czech experiment it tested very resistant on a species level, whereas in the Dutch experiment a cultivar of this species (‘Emma’s Gold’) tested rather susceptible.
Within the highly susceptible species *F. excelsior* (European ash) the cultivars tested clearly differed in susceptibility to ash dieback, ranging from very susceptible to moderately susceptible, with especially ‘Diversifolia’, ‘Eureka’, ‘Geessink’ and ‘Allgold’ performing better than most other cultivars.

The results of the survey of urban ashes in Czech Republic are very much in line with those of the field screening experiments. The *Fraxinus* species most damaged is *F. excelsior* and most of its cultivars have been badly affected too. The exception is *F. excelsior* 'Nana', the results of which were very good, comparable to those of American *Fraxinus* species (*F. pennsylvanica* and *F. americana*). The species least affected in this survey was *F. ornus*. Selected cultivars and species will be tested for resistance in situ in the future with the aim of possible application as resistant replacement for seriously affected cultivars.
Similarly the survey of *F. excelsior* trees in the urban areas of Latvia identified over 50 healthy individuals, probably more resistant to infection by *H. fraxineus*, for further testing in future.
Additionally, the results of the survey in Latvia indicate the important influence of nutrition, soil and environmental conditions on the condition of diseased ash trees. Combination of all or some of these factors might contribute to the death of the trees, making it difficult to determine the main cause of death. Concerning nutrition especially sulpher deficiency was identified as an important factor that needs further research.

Summarizing, the results show that *Fraxinus* species clearly differ in susceptibility to Ash dieback and that within species cultivars may differ significantly in susceptibility to the disease. Susceptibility of a large array of *Fraxinus* species and cultivars has been characterized. Unfortunately European or common ash (*Fraxinus excelsior*) cultivars tested vary from highly to moderately susceptible with only some of the cultivars performing slightly better. On a species level large differences were observed with some species being very susceptible whereas some others tested much less susceptible. Especially *F. ornus*, *F. americana*, *F. pennsylvanica* and *F. mandchurica* appeared much less susceptible. These results provide important information for management of Ash dieback in existing urban green as well as for limiting disease risk in new plantations.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

Cleary M., Arhipova N., Gaitnieks T., Stenlid J., Vasaitis R. (2013). Natural infection of Fraxinus excelsior seeds by Chalara fraxinea. Forest Pathology. 43. 83-85.

Coker TLR, Rozsypálek J, Edwards A, Harwood TP, Butfoy L, Buggs RJA; 2019. Estimating mortality rates of European ash (*Fraxinus excelsior*) under the ash dieback (*Hymenoscyphus fraxineus*) epidemic. *Plants, People, Planet*, 2019;1:48–58. https://doi.org/10.1002/ppp3.11 Forestry Commission, 2013. NFI survey of *Chalara Fraxinea*. NFI Report 15/02/2013, 8pp.

Cooper R.M., Williams J.S. (2004). Elemental sulphur as an induced antifungal substance in plant defence. Journal of Experimental Botany, Volume 55, No. 404.

Kenigsvalde, K., Arhipova, N., Laiviņš, M., Gaitnieks, T. (2010). Fungus Chalara fraxinea as a causal agent for ash decline in Latvia. Mežzinātne. Forest Science 21(54): 110-120.

Kopinga J; Vries de S; 2013. Differences in susceptibility to *Chalara fraxinea* (twig dieback of ash) of selections of Common ash (*Fraxinus excelsior*) in the Netherlands : report of the observations and results of 2012 (in Dutch). CGN rapport 26. 32 pp.

Kowalski T and Holdenrieder O; 2009. Pathogenicity of *Chalara fraxinea*. For. Path. 39 (2009) 1–7.

Kräutler K, Treitler K and Kirisits T; 2015. *Hymenoscyphus fraxineus* can directly infect intact current-year shoots of *Fraxinus excelsior* and artificially exposed leaf scars. For. Path. 45: 274–280.

Lösing H; 2012. Einige *Fraxinus*-Arten trotzen dem Eschensterben. Deutsche Baumschule 2012(3):40-42.

McKinney LV *et al*.; 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (*Oleraceae*) to *Chalara fraxinea* (*Ascomycota*): an emerging infectious disease. Heredity. 106: 78-797

Pliura A, Lygis V, Suchockas Y, Bartevicius E; 2011. Performance of twenty four European *Fraxinus excelsior* populations in three Lithuanian progeny trials with a special emphasis on resistance to *Chalara fraxinea*. Baltic Forestry 17:17–33

Schnug E and Haneklasu s; 1998. Diagnosis of Sulphur nutrition. P. 1-38 in: E. Schnug (ed.), Sulphur in Agroecosystems, Kluwer Academic Publishers.

Stener L-G; 2012. Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* in southern Sweden. Scandinavian Journal of Forest Research.

Tomiczek C; 2014. Invasive pests and diseases on trees – new threats around the corner. Jahrbuch der Baumpflege 2014:146-153.

UK Forestry Commission; 2016. Managing Chalara Ash Dieback in England. https://www.forestry.gov.uk/pdf/National_Chalara_leaflet_Feb_2016.pdf/$file/National_Chalara_leaflet_Feb_2016.pdf