

# Estimation of Ash Mortality Induced by *Hymenoscyphus fraxineus* in France and Belgium

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## Summary

Ash dieback induced by *Hymenoscyphus fraxineus* has emerged as one of the most serious health problem for European forests in the last ten years. However, precise estimation of the mortality induced by the pathogen is still scarce and this hampers management of affected stands. In this work, we used data of several surveys done since 2010 in France and Belgium to estimate the mortality rate associated with ash decline depending on the time of the pathogen presence in the area; for that a 2 steps procedure was used. First, we did an estimation of the frequency and severity of collar lesions associated with *H. fraxineus* depending on the length of the pathogen presence and for 2 trees size classes (lower or higher than 25 cm dbh). Then the annual mortality rate was estimated depending on collar lesion severity, dbh class (lower or higher than 25 cm) and time since pathogen presence. The global mortality induced by *H. fraxineus* was computed from those 2 types of data by a bootstrap approach. Additionally one survey observing young stands was used from which mortality was computed directly. We find that if mortality is drastic in very young ash stand affected by *H. fraxineus* (less than 5 cm dbh), with annual mortality reaching 35% 5-6 years after arrival of the pathogen in the stand, it is much more moderate for trees with dbh above 25 cm, with annual mortality reaching 3.2% after 8-9 years of pathogen presence. Annual mortality rates are intermediate for trees in the 5-25 cm dbh class and reached 10-11% after 8-9 years of pathogen presence.

**Key words:** Ash dieback, Fraxinus, mortality, invasive pathogen

## Introduction

Ash dieback has emerged as one the most serious health problem for European forests in the 10 last years. The disease is induced by *Hymenoscyphus fraxineus*, an invasive pathogen that originated from East Asia (Gross et al. 2014b). Ash dieback was first reported in the early nine-

ties in Poland and since it spread toward throughout Europe (Gross et al. 2014a, McKinney et al. 2014). In Western Europe, it reached France in 2008 and Belgium in 2010 (Chandelier et al. 2016, Husson et al. 2011). Ash dieback has a life cycle of a foliar disease, overwintering on the leaf rachis in the litter and producing apothecia from the colonized rachis from June to August (Gross et al. 2014a).

However, the pathogen is able to infect shoots from the colonized leaves and causes extensive shoot dieback during the winter season (Gross et al. 2014b). *H. fraxineus* has also been shown to induce extensive lesions in the inner bark of the collar area (Husson et al. 2012). These lesions are secondarily colonized very quickly by *Armillaria* species (Skovsgaard et al. 2010, Bakys et al. 2011, Husson et al. 2012, Enderle et al. 2013, Chandelier et al. 2016), which may explain why root rot has been frequently reported on ash affected by *H. fraxineus*. In addition, it has been noticed repeatedly that deterioration of ash health status is strongly associated with the development of collar lesions, whether those are supposed to be associated with *H. fraxineus* or with *Armillaria* species (Bakys et al. 2011, Chandelier et al. 2016, Lenz et al. 2016).

*H. fraxineus* has been reported to threaten the future of ash and its associated ecosystem in Europe (Lygis et al. 2011, Pautasso et al. 2013, Löhmus and Runnel, 2014). However, precise estimations of the mortality induced by the pathogen are still scarce. In young ash seedlings / saplings, high mortality rate have been reported (Pliura et al. 2011, Koltay, 2012, Enderle et al. 2013). Data is far less available in mature stands. Löhmus and Runnel (2014) report a mortality of about 50% in 4 years in a mature ash stands while Rosenvald *et al* (2015) report annual mortality rate of about 2-5% per year for solitary ash trees retained after timber harvesting. By contrast, Keßler *et al* (2012) report very little mortality associated with ash dieback between 2008 and 2010 in their survey of the disease impact in Austria. The lack in precise knowledge on the level and timing of mortality associated with ash dieback clearly hampers the management of affected stands.

The aim of this work was to use the available surveys on ash dieback impact that were done in France and Belgium to derive estimates on the level of mortality in relation to the time of *H. fraxineus* presence in the area. As data on very young stands are less scarce, the main effort was targeted to mature and pole size trees.

## Material and methods

The study was based on several surveys done in France and Belgium over 1-6 years to monitor the health of ash trees affected by *H. fraxineus*.

### *Plots observed over more than 1 year to monitor mortality*

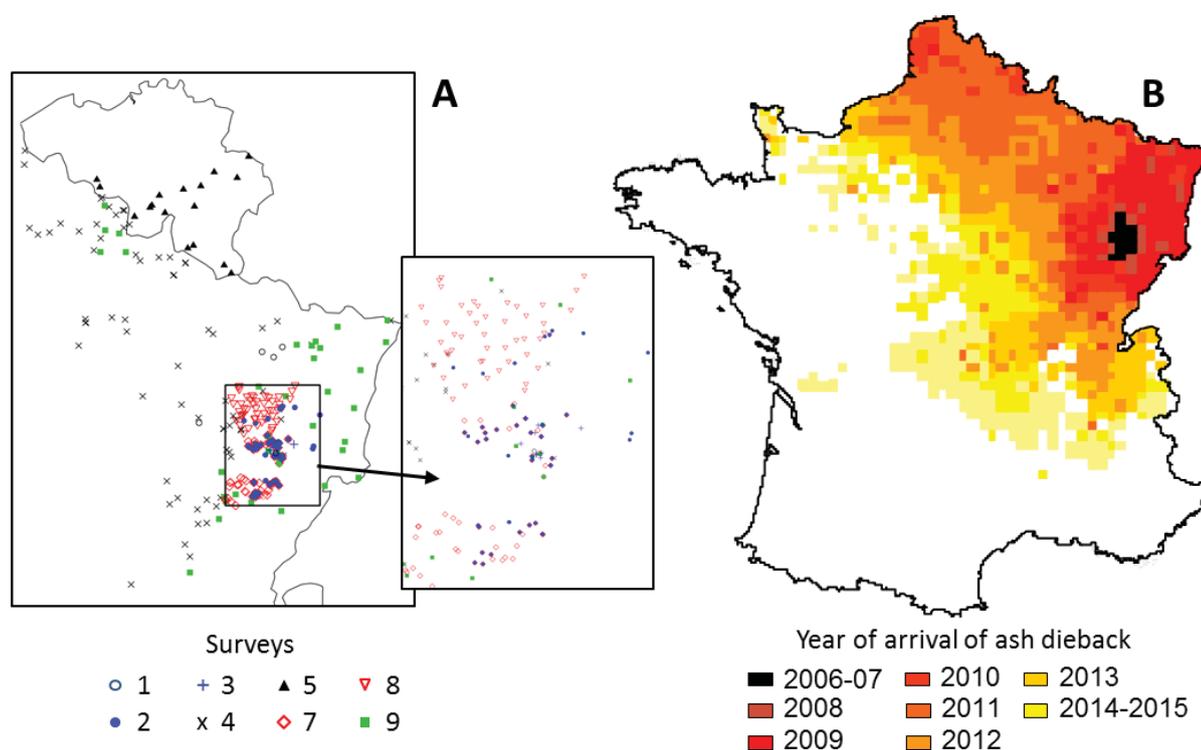
**Survey 1.** Six young ash stands originating from seeding (2-4 m height) were sampled in NE France and monitored for 3-5 years (see Table 1). The Seichamps stands, close to Nancy, originated from seeding in an abandoned field and contained only ash saplings with a very high density (30 000 stem.ha<sup>-1</sup>). The second, located Gremecey (Moselle) was an oak/maple plantation where ash settled by seeding with a density of 1 400 stem.ha<sup>-1</sup>. Chaumont (Haute-Marne), Meurcourt (Haute-Saône) and the 2 stands (P19 and P36) of Pompey (Meurthe-et-Moselle) are forest stands originating from seeding with a mixture of ash and oak/beechness and has a density of ash of 8 300, 1 260 and 9 400 stems.ha<sup>-1</sup>, respectively. Depending on density, quadrats of 1-20m<sup>2</sup> were regularly spaced in each stands and all ash seedlings present in the quadrats were marked and surveyed. Altogether, the number of rated seedlings was of 174 in Seichamps, 203 in Gremecey, 555 in Chaumont, 300 in Meurcourt, 1619 in P19 and 280 in P36.

**Survey 2.** This survey was done in 2011-12 in Haute-Saône and Vosges area, in the initial focus of ash dieback in NE France. The mean diameter at breast height (dbh) of the selected trees ranged from 8 to 38 cm depending on the plot (3 plots with mean dbh>25cm and 39 with mean dbh<25cm).

**Survey 3** was set up in the area of Haute-Saône infected since 2008 with a very high disease severity reported since 2010 (Husson et al. 2011). The aim was to monitor the health of ash trees that remained healthy in 2010. Eighty nine ash trees with no crown or collar symptoms as well as 163 of their dieback affected neighbor were selected and

**Table 1.** Description of the surveys

Survey	Nb plot	Nb trees	Years	Location	Age (years)	dbh range (cm)	Reference
1	6	3651	2012-16	Grand Est, Franche- Comté	3-10	2-7	-
2	42	1023	2012-13	Haute- Saône, Vosges	-	8-38	Marçais et al. (2016)
3	13	252	2012-15	Haute-Saône	-	12-45	-
4	40	648	2010-15	N and NE France	-	10-60	-
5	17	288	2013-15	Wallonia (Belgium)	-	16-46	Chandelier et al. (2016)
6	1	775	2012-15	Devecey (Doubs)	19	15	Muñoz et al. (2016)
7	60	3265	2010	Haute-Saône	-	10-25	Husson et al. (2011)
8	48	1350	2013	Vosges	-	6-50	-
9	87	2610	2012-15	N and NE France	-	-	-



**Figure 1.** Localisation of studied plots (A) and map of *H. fraxineus* presence (B, source DSF)

marked in 2012 and monitored until 2015. The mean dbh of selected trees ranged 12 to 45 cm depending on the plot (10 plots with mean dbh < 25 cm and 3 plots with mean dbh > 25 cm).

**Survey 4-5.** These surveys were set up in 2010 by the Département de la Santé des Forêts (DSF) and 2013 by the Department Natural Ecosystem and Agriculture of Wallonia to document the evolution of ash dieback at the tree-scale. From 11 to 20 ash trees were selected and marked in 57 stands scattered in the NE and Northern France in 2010 (24 stands) and 2011 (16 stands) and in Wallonia (17 stands). They were annually monitored for health status until 2015. Mean dbh of the selected trees ranged from 10 to 60 cm depending on the plot with 38 stands having a mean dbh over 25 cm.

**Survey 6** was performed in a plantation of ash half sibling families. We did not use the available genetic information in this study. We used data for 2012-2015 where information on the severity of collar girdling by *H. fraxineus* was available.

Surveys 1-6 were used to estimate mortality. In survey 1-6, the trees were separated in 3 categories according to mean stand dbh. Altogether, ash rated in each category was of 3141 seedlings of 1-5 cm dbh (survey 1), 2280 ashes of 5-25 cm dbh (survey 2, 3, 4, 5 and 6) and 707 ashes of 25-60 cm dbh (survey 2, 3, 4, and 5).

#### *Plots observed for just 1 year for estimation of disease severity*

**Survey 7 and 8** were both designed similarly by the DSF with the aim to estimate the local incidence of *H. fraxineus* (see table 1).

**Survey 9** was derived from the DSF data base by extracting all the report of the observation strategy “DE” that dealt with *H. fraxineus* on ash. This strategy is aimed at quantifying the severity of disease problems by detailed counts of trees in severity classes. We used all the report from 2012 to 2015 that documented the severity of collar cankers associated with *H. fraxineus* and for which an approximate mean stand dbh could be retrieved.

Survey 2-9 were used to estimate the evolution of tree health and in particular of the severity of collar canker, which represents 310 stands. The location of the different surveyed stands is showed in Figure 1a. The sampling is dense in the area of NE France where ash dieback was reported for the first time in 2008 but covers north-eastern and northern France as well as southern Belgium. The surveyed stands were separated in 2 size class (lower and higher than 25 cm mean stand dbh). The small tree size class represent 209 stands (7791 ash trees) while the large tree size represent 101 stands (2420 ash trees).

### Survey procedure

Trees were rated annually between June and August, depending on the survey, for both crown status and collar canker severity. The crown status was rated as 0, 0-5% defoliation, 1, 5-25% defoliation, 2, 25-50% defoliation and 3, over 50% defoliation. Some surveys included a fifth category (more than 75% defoliation) that was not used because it was not available for all survey. The basal canker was rated for the %collar girdled in 5 categories: as 0, 0-5% girdling, 1, 5-25% girdling, 2, 25-50% girdling, 3, 50-75% girdling and 4, over 75% girdling. In some survey, the total collar circumference as well as the sum of basal lesion widths were both determined and the % collar girdled was computed from those data (surveys 2, 3, 6). In other, the rating was done visually directly in the stands (surveys 4, 5, 7-9). In survey 1, young ashes were rated annually as healthy, affected by *H. fraxineus* or dead. No basal canker was looked at in these regenerations.

For survey 1-8, dbh was determined at least once for each tree observed. For survey 9, the approximate stand dbh was retrieved from the remark done by the DSF observers; only reports where this was possible were included.

### Assessment of ash dieback time of arrival in the area

The length of *H. fraxineus* presence in the area is derived from the DSF. The year of first report of *H. fraxineus* is available for all France by quadrats of 16 x 16 km (Fig. 1b). Unpublished observations made in NE France at the time of *H. fraxineus* arrival suggest that the local spread of the pathogen is extremely fast and that it can be assumed that, at a location, all stands are infected within 1-2 years. We thus assumed that *H. fraxineus* was present in a stand in the year it was reported for the first time in the DSF database in the 16 x 16 km quadrat where it is located. Ash dieback was reported in France for the first time in 2008 in the north of Haute-Saône. However, it is very likely that the disease had been present there since few years when first reported because the severity observed in the area in 2008 was already very drastic. Moreover, it is reported in Husson et al (2011) that the first collar cankers could be observed in the area in 2007 while this type of symptoms is usually observed only 2-3 years after arrival of *H. fraxineus* in a stand. Thus, we adapted the year of first presence of *H. fraxineus* in the area. To remain conservative, we thus supposed that the 4 quadrats of north Haute-Saône / south Vosges were colonised in 2006 and the 6 adjacent quadrats in 2007. The time of *H. fraxineus* presence was determined as the year of observation minus the year of first disease report in the quadrat. Thus we may observe 9 years post-infection (2006+9 = 2015). The modified map of *H. fraxineus* presence in France is shown in Fig. 1b.

### Data analysis

The influence of crown and collar status on ash tree mortality was studied by mixed logistic regression with the function `glmer` of R library `lmer` using data of survey 2-6. The model included the log of time since first report of *H. fraxineus* in the area, crown status as 0-1, 2 or 3, collar status as 0-1, 2, 3, 4 and age category which took 2 levels (mean stand dbh lower than 25cm or above 25 cm) as fixed factor. Two different random factors were tested in a preliminary step, a tree factor and a stand factor. The stand factor was retained for further analysis.

In order to estimate the mortality rate induced by *H. fraxineus* depending on the time of pathogen presence, we proceeded in 2 successive steps, with first i. an estimation of the severity of collar girdling by the pathogen at different years after first report of the ash dieback in the area, using for that survey 2-9 and then ii. Estimation of the mortality per collar girdling classes 0-4, using the data of surveys 2-6. The 2 estimations were done for each of the 2 tree size class (mean stand dbh lower or above 25 cm) and of the 2 periods (less than 5 years of *H. fraxineus* presence in the area or over 5 years).

An underlying hypothesis is that canker severity depends only on tree size and on time since *H. fraxineus* arrival in the stand. Others factors influence canker severity such as site humidity (Husson et al. 2011, Marçais et al. 2016) and likely other local conditions (forest/landscape, road side, river side, site vegetation or understory). But we chose to neglect these site factors because they are not likely to play a major role at the regional level. Tree size was kept because it has important management consequences. Additionally, we were not able to take into account both crown status and collar status in the previous year because one of the major sources of data, the survey 9, gives separate counts for distribution of trees in the crown decline classes and in the collar girdling classes. Collar girdling was chosen for the estimation because it appears more tightly related to mortality.

The estimates of frequency in collar girdling classes, mortality per girdling classes and global mortality per size categories derive from bootstrap samples (5000 samples). The resampling occurred in 2 steps. First, a number of stands equal to the observed number was sampled with replacement in the list of observed stands. Then, within each selected stands, a number of trees equal to the number of trees observed was sampled with replacement in the list of observed trees. For each resampled dataset, the mortality for each canker girdling classes was computed for the 2 dbh classes and the 2 periods of *H. fraxineus* presence. The evolution of canker girdling class relative frequency was computed for each dbh class and each year following *H. fraxineus* arrival in the area. The mortality per year following *H. fraxineus* presence and per dbh class was then computed as:

$$[1] \quad \sum CS_i \times P_{dead,i}$$

with  $CS_i$  the frequency of canker girdling class  $i$  and  $P_{dead,i}$  the mortality associated with the canker girdling class  $i$ . The mean and the 0.025 and 0.075 quantiles were then computed.

For young ash stands (survey 1), the mortality was directly estimated from the data of survey 1, using the bootstrap strategy in 2 steps previously described (sampling with replacement of the stands and then in a second step of the trees in the sampled stands). The mortality was then computed from each resampled dataset.

## Results

### *Ash mortality and tree status in the previous year*

In a preliminary step, logistic regression was done with a stand and a tree random factor. The variance associated with the tree factor is very small ( $1.2 \times 10^{-12}$ ) and the deviance drop associated with this random factor is not significant (Chisq = 0.5,  $p = 0.480$ ) and thus only the stand random factor has been retained in the analysis. The stand factor is associated with a variance of 0.574 and a significant deviance drop (Chisq = 24,  $p < 0.001$ ).

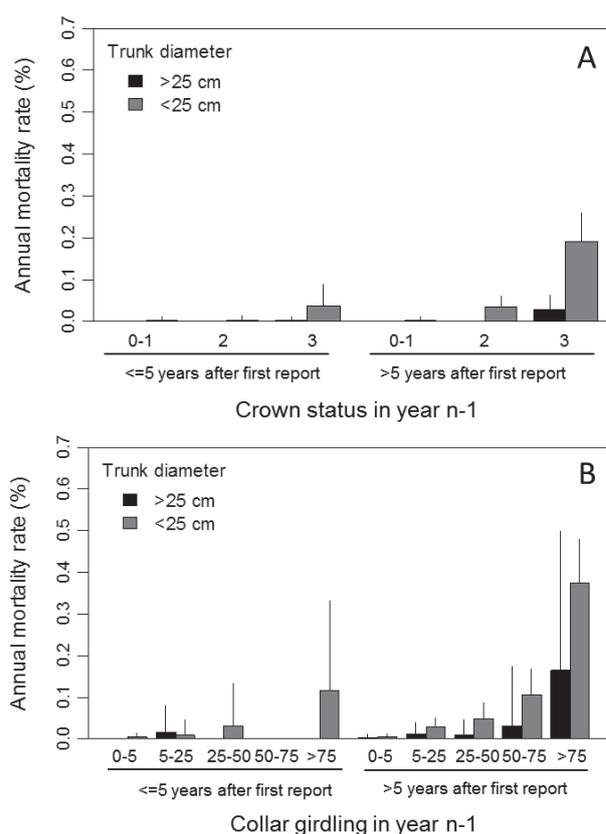
Table 2 gives the result of the logistic regression. All main factors, i.e. time since *H. fraxineus* arrival in the area, tree size class, level of crown decline and of collar girdling by the pathogen are all significantly associated with mortality. By contrast, interactions of age with level of crown decline and of collar girdling and interaction between crown decline and collar girdling are not significant (table 2). Figure 2 shows the evolution of ash mortality with crown status and collar girdling for the 2 tree size classes and the 2 length of *H. fraxineus* presence in the stand. Mortality in the 4 years following arrival of *H. fraxineus* in the area is much lower than 5-9 years post pathogen arrival. Mainly trees which have developed collar lesions die during this early period. In the second half of the 9 year period, substantial mortality can be observed for the more severe crown decline and collar girdling classes. Mortality was also much more severe for ash trees in the 5-25 cm dbh size class than in stands with trees over 25 cm dbh.

Collar girdling appears to have a size effect higher than crown decline on mortality: after 5 years of *H. fraxineus* presence, a mortality for trees in the 5-25 cm dbh class with the most severe collar girdling is of 36% [26, 47] compared to 20% [13, 27] for the most severe crown die-back class. For that reason, collar girdling by *H. fraxineus* was selected for the following part of the work.

### *Evolution of ash health status and mortality with time of presence of H. fraxineus*

The severity of collar infection increases steadily with the number of years of *H. fraxineus* presence in the area (Fig. 3), while the status of the crown deteriorates

(Fig.4). For the 2 types of symptom, the status deteriorates earlier and stronger for ash in the 5-25 cm dbh class compared to those in the >25 cm dbh class. In a first step, collar girdling remained lower in stands with mean dbh over 25 cm compared to dbh lower than 25 cm. In particular, almost no tree with collar girdling of more than 75% occurred in stands with a mean dbh >25 cm until the seventh year of *H. fraxineus* presence while in stands of smaller trees, ash with a collar girdled more than 75% appeared after only 5 years of the pathogen presence. After 8 years of disease presence, collar girdling did catch up in stands of large trees: we then



**Figure 2.** Annual mortality rate of chalara infected ash and status of the tree in the previous year for 2 ash size classes and 2 times since arrival of *H. fraxineus* in the area. A. Crown decline classes. B. Severity of collar girdling (based on bootstrap samples of 5000)

**Table 2.** Mixed logistic regression analysis of ash mortality

	Df	LR Chisq	P value
Log(time of presence) <sup>a</sup>	1	23.8	<0.001
Tree size (dbh) <sup>b</sup>	2	15.7	<0.001
Crown status <sup>c</sup>	2	118.1	<0.001
Collar girdling <sup>d</sup>	3	93.7	<0.001
Crown status * Tree size	2	0.3	0.99
Collar girdling * Tree size	3	1.2	0.75
Crown status * Collar girdling	6	5.4	0.50

<sup>a</sup> nb year since *H. fraxineus* arrival in the 16 x 16 quadrat

observed a frequency of ash tree with >75% of the collar girdled of about 15-20 % in stands with mean dbh<25 cm and of about 10-15% in more aged stands (>25 cm dbh). This category is the one with really high mortality (Fig. 2).The decrease in frequency of healthy looking crown (rated as 0 or 1) occurs also earlier than increase in the frequency of trees with collar lesions (Fig. 3 and 4). However, a very strong variability existed between stands in the development of collar lesions. About 17% of the stands present no collar canker after 6 years while severe collar infection are present in 20% of the stands after only 3 years (over 75% collar girdled). Altogether, after 8-9 years of *H. fraxineus* presence in the area, about 80-90% of the ash trees show some basal lesions although significant infection with over 25% of the collar girdled then represented about 50-60 % of the tree.

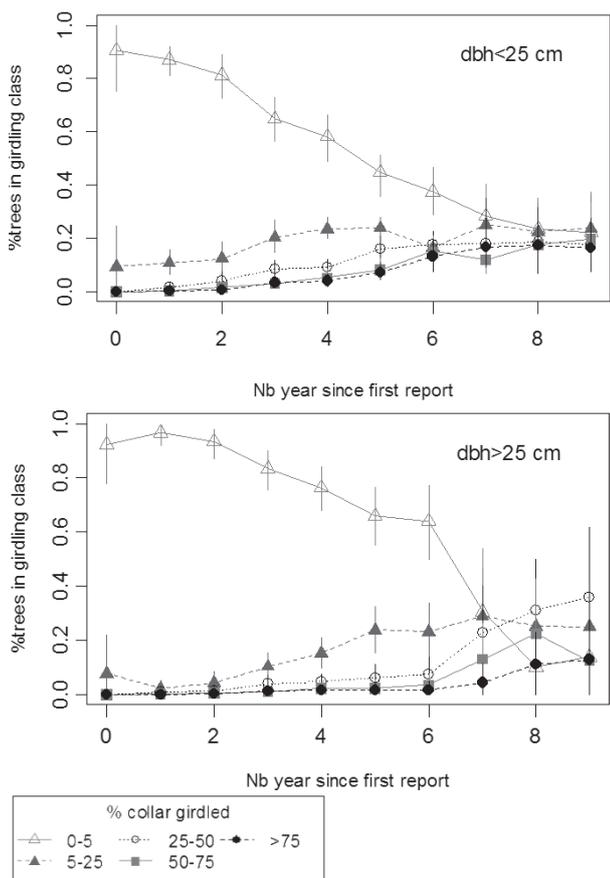
Unlike collar girdling, evolution of crown status is basically similar between the two tree size classes from the second/third year after *H. fraxineus* arrival (Fig. 4). Asymptomatic ash trees frequency (0-5% defoliation) tend to stabilize at 1% after 8 years of disease presence while the most

severe crown dieback class is still increasing and represent approximately 40-50% 9 years after the pathogen discovery.

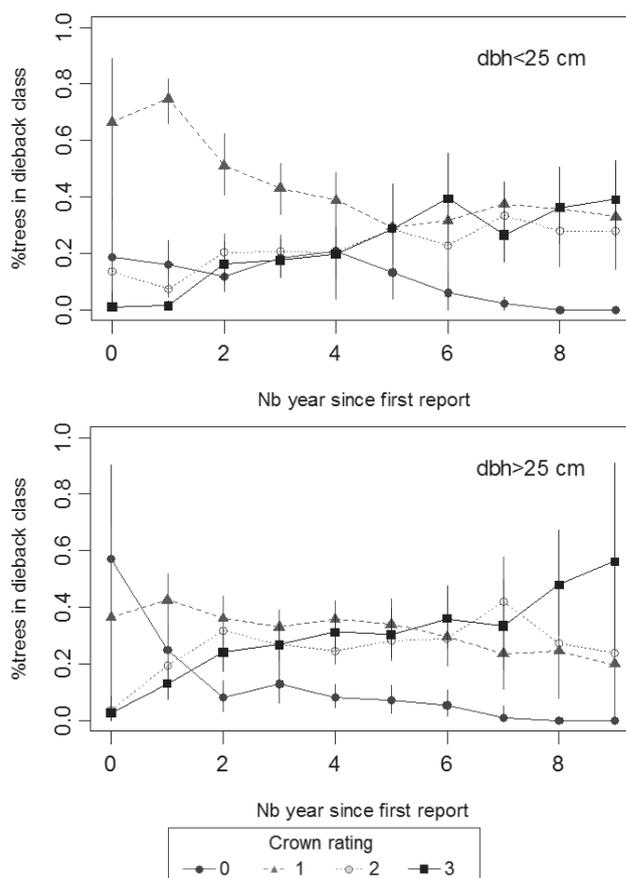
Figure 5 gives the evolution of annual mortality for the 3 age classes. Annual mortality is drastic in the regenerations, quite strong in stands with mean dbh less than 25 cm but remains low in large dbh stands, with about 3.2% 9 years post infection. Mortality appears to reach a plateau in stands of all size categories. This is the most noticeable in stands of 5-25 cm mean dbh with mortality remaining in the 10-11% range (confidence interval of 8-19 %) for times of *H. fraxineus* presence of more than 6 years.

### Discussion

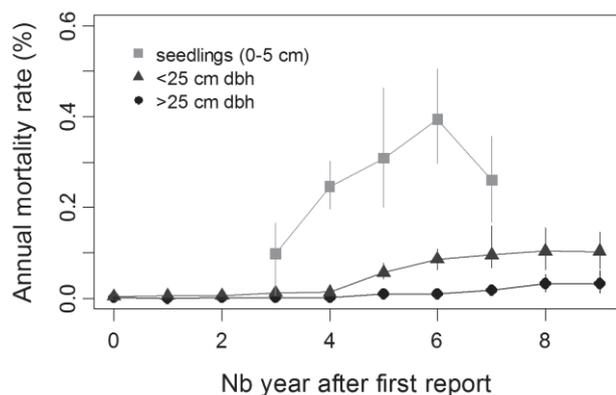
This work quantified mortality due to *H. fraxineus* in ash stands in the 9 years following the arrival of the pathogen in France and Belgium. As has been already reported, mortality is very high in young ash seedlings and saplings and to a lesser degree in the 5-25 cm dbh class (respectively 35% and 11% after 6 year of *H. fraxineus* presence). By



**Figure 3.** Evolution of level of collar girdling by *H. fraxineus* with time of pathogen presence in the area (based on bootstrap samples of 5000)



**Figure 4.** Evolution of the crown status of ash with time of *H. fraxineus* presence in the area (based on bootstrap samples of 5000)



**Figure 5.** Evolution of mortality linked to *H. fraxineus* after introduction of the pathogen in an area (based on bootstrap samples of 5000)

contrast, we found that mortality of large ash of more than 25 cm dbh remains moderate, at 3.2% per year. A large part of the mortality can be explained by the status of the tree in the previous year, collar girdling by the pathogen and crown decline.

Mortality was influenced additively by crown status and by collar girdling. Both appeared to play an important role. For practical reasons, it was not possible to take these 2 factors into account in the final estimation of the mortality rate. The 2 symptoms have been shown to be tightly correlated at a stand level (Bakys et al. 2011, Husson et al. 2012, Chandelier et al. 2016). Moreover it was suggested that strong crown deterioration often follows the development of severe collar infection (Chandelier et al. 2016). Thus, taking into account only collar status in the computation of global mortality maybe a good compromise although it would probably have improved the estimate to also take into account the crown status. It has been suggested that the development of collar lesions necessitate a strong inoculum from apothecia at the base of the tree and thus appears only after few years of *H. fraxineus* presence in the stand (Chandelier et al. 2016). In agreement with this, we observed in this work an increase in the frequency and severity of collar lesions only 2-3 after the first report of the pathogen in the area.

Our results showed that mortality was strongly influenced by tree size, i.e. low to moderate in large trees, high in pole size ash and drastic in young regenerations. The lower *H. fraxineus* impact on large trees compared to young ash and seedling has already been reported by several authors (Husson et al. 2012, Chandelier et al. 2016, Lenz et al. 2016). The high mortality rate in young stands is in agreement with what was shown in Central and Eastern Europe, in stands originating either from seeds or from planting (Pliura et al. 2011, Koltay, 2012, Enderle et al. 2013, McKinney et al. 2014). By contrast, low mortality rate of mature trees affected by *H. fraxineus* have also been

reported by Lenz *et al* (2016). The mortality rate of 3.2% observed in this study for large trees after 8-9 years of the pathogen presence is comparable to the figure reported by Rosenvald *et al* (2015) for solitary ash trees retained after timber harvesting in Estonia.

A large between-stands variability in mortality rate has been observed. Part of the variability is in the severity of the ash dieback symptoms and in particular in the severity of tree collar girdling by the pathogen. The nature of site and stand factors that could explain this variability are beyond the scope of this work. Nevertheless, it was shown that canker frequency and severity could be influenced by both site moisture and by tree size (Husson et al. 2012, Chandelier et al. 2016, Marçais et al. 2016). In agreement, we observed in this work that stands with large trees (>25 cm mean dbh) were affected less and later by collar canker. However, the severity of ash dieback crown and collar symptoms did not explain all mortality as both the stand random factor and as the duration of the pathogen presence in the area explained a significant part of the variability as well.

The difference in tree mortality at a given crown/collar status may be linked to the capacity of the trees to cope with stresses. Trees exposed to *H. fraxineus* suffer chronic infection with high mortality of shoots produced in the previous year. Their ability to cope with this may depend on the site suitability for ash, on their past history and on their exposure to secondary pest such as *Armillaria*. Indeed, mortality is a complex process not yet fully understood. It has been shown that trees may die because a stress such as defoliation or drought has depleted their carbohydrate reserves, potentially leading to carbon starvation (Marçais and Bréda, 2006, Bréda et al. 2006, Sevanto et al. 2014). The vigor of the tree at the time of first infection and its past history including the duration of exposure to *H. fraxineus* both are likely to impact its carbohydrate reserves level and could determine its ability to survive at a given crown / collar status. The fact that the stand random factor was associated with a part of mortality variability much higher than the tree random factor may indicate that individual tree history may be less important than factors acting at the stand level such competition level or site factors. For that reason, in the bootstrap procedure to estimate mortality, we included a first step of resampling of the stands.

The annual mortality appeared to reach a plateau after 6-7 year of *H. fraxineus* presence, especially for pole size trees (5-25 cm dbh). This may be favorable sign for future although annual mortality stabilized at a very high level. Possible future evolution depends on several factors. First, the severity of collar lesions also tended to stabilize after 6-7 years, especially for trees of the 5-25 cm dbh class. This probably happens because the trees the more severely girdled by *H. fraxineus* suffer very high mortality

and are constantly being removed from the population. As it has been showed that genetically based resistance toward the pathogen exists in *Fraxinus excelsior* (McKinney et al. 2010, Pliura et al. 2011) and that resistance to collar infection has also be demonstrated to exist (Muñoz et al. 2016), one may anticipate a progressive increase in the resistance level of the population with time. This might induce a decrease of the mortality rate after a prolonged period of infection. Then, the fact that mortality at a given health status depends on time since infection is not favorable as it may indicate that trees are progressively weakened and more prone to die. It is thus impossible to extrapolate the results of the study to the future.

The consequences of our results for long term impact of ash dieback are important. As was already reported by forest managers throughout Europe, the observed levels of mortality in the stands of 5-25 cm dbh (pole size) jeopardize all possibility of ash stand management. After few year of such high mortality, about half of the stand ash trees are dead. Even a level of 3.2% annual mortality in mature stands with reduced density where very few trees usually dies will preclude any management; moreover a majority of surviving trees are in poor health conditions. The ecological consequences may be less clear-cut. Very pessimistic prospects have been predicted for the ash ecosystem (Pautasso et al. 2013). However, this work shows that affected mature trees do not die fast and that ash dieback may not threaten that much these ecosystems on the short term. By contrast, the impact on ash young stands, especially those with less than 5 cm dbh is drastic and establishing the ash stands of the future appears as the main challenge. The work reported by Lygis et al (2011) well illustrates this point. Ash dieback has been managed in the first years in Lithuania before the widespread recognition that the disease was caused by an invasive pathogen and that resistance to the pathogen was present. In this situation, while ash dominated the stands in the previous generation, it became a minor component in the regeneration. Thus retaining ash as a significant component of the new generation stands will require a proactive management. In particular, selecting both the seed trees and the regenerating seedlings on their ability to tolerate the *H. fraxineus* infection is important as it has been pointed out by Mc Kinney et al. 2014. Some studies already started to address this issue (Rosenvald et al. 2015).

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### References

- Bakys R., Vasiliauskas A., Ihrmark K., Stenlid J., Menkis A., Vasaitis R.** 2011. Root rot, associated fungi and their impact on health condition of declining *Fraxinus excelsior* stands in Lithuania. *Scandinavian Journal of Forest Research* 26:128–135.
- Bréda, N., Huc, R., Granier, A. and Dreyer, E.** 2006. Temperate Forest Trees and Stands under Severe Drought: A Review of Ecophysiological Responses, Adaptation Processes and Long-Term Consequences. *Annals of Forest Science* 63: 625–44.
- Chandelier, A., Gerarts, F., San Martin, G., Herman, M. and Delahaye, L.** 2016. Temporal Evolution of Collar Lesions Associated with Ash Dieback and the Occurrence of *Armillaria* in Belgian Forests. *Forest Pathology* 46: 267–379.
- Enderle, R., Peters, F., Nakou, A. and Metzler, B.** 2013. Temporal Development of Ash Dieback Symptoms and Spatial Distribution of Collar Rots in a Provenance Trial of *Fraxinus excelsior*. *European Journal of Forest Research* 132: 865–876.
- Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V. and Sieber, T.N.** 2014a. *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. *Molecular Plant Pathology* 15: 5–21.
- Gross, A., Hosoya, T. and Queloz, V.** 2014b. Population structure of the invasive forest pathogen *Hymenoscyphus pseudoalbidus*. *Molecular Ecology* 23: 2943–2960.
- Husson, C., Caël, O., Grandjean, J.P., Nageleisen, L. and Marçais, B.** 2012. Occurrence of *Hymenoscyphus pseudoalbidus* on infected ash logs. *Plant Pathology*, 61: 889–895.
- Husson, C., Scala, B., Caël, O., Frey, P., Feau, N. and Marçais, B.** 2011. *Chalara fraxinea* is an invasive pathogen in France. *European Journal of Plant Pathology* 130: 311–324.
- Keßler, M., Cech, T.L., Brandstetter, M. and Kirisits, T.** 2012. Dieback of ash (*Fraxinus excelsior* and *Fraxinus angustifolia*) in Eastern Austria: disease development on monitoring plots from 2007 to 2010. *J. Agric. Ext. Rural Dev.* 4: 223–226.
- Koltay, A.** 2012. *Chalara fraxinea* Incidence in Hungarian Ash (*Fraxinus excelsior*) Forests. *Journal of Agricultural Extension and Rural Development* 4, doi:10.5897/JAERD12.058.
- Lenz, H., Bartha, B., Straßer, L., and Lemme, H.** 2016. Development of Ash Dieback in South-Eastern Germany and the Increasing Occurrence of Secondary Pathogens. *Forests* 7: 41.
- Löhmus, A. and Runnel, K.,** 2014. Ash Dieback Can Rapidly Eradicate Isolated Epiphyte Populations in Production Forests: A Case Study. *Biological Conservation* 169: 185–88.

- Lygis, V., Bakys, R., Gustiene, A., Burokiene, D., Matelis, A. and Vasaitis R.**, 2014. Forest Self-Regeneration Following Clear-Felling of Dieback-Affected *Fraxinus Excelsior*: Focus on Ash. *European Journal of Forest Research* 133: 501–10.
- Marçais, B. and Bréda, N.** 2006. Role of an opportunistic pathogen in the decline of stressed oak trees. *Journal of Ecology* 94: 1214-1223.
- Marçais, B., Husson, C., Godart, L. and Caël, O.** 2016. Influence of site and stand factors on *Hymenoscyphus fraxineus* induced basal lesion. *Plant Pathology* DOI 10.1111/ppa.12542.
- McKinney, L.V., Nielsen, L. R., Collinge, D.B., Thomsen, I.M., Hansen, J.K. and Kjaer, E.D.** 2014. The ash dieback crisis: genetic variation in resistance can prove a long-term solution. *Plant Pathology* 63: 485–499.
- McKinney, L.V., Nielsen, L.R., Hansen, J.K. and Kjær, E.D.** 2010. Presence of Natural Genetic Resistance in *Fraxinus excelsior* (Oleraceae) to *Chalara fraxinea* (Ascomycota): An Emerging Infectious Disease. *Heredity* 106: 788–97.
- Muñoz, F., Marçais, B., Dufour, J. and Dowkiw A.** 2016. Rising out of the ashes: additive genetic variation for susceptibility to *Hymenoscyphus fraxineus* in *Fraxinus excelsior*. *Phytopathology* DOI: 10.1101/031393.
- Pautasso, M., Aas, G., Queloz, V. and Holdenrieder, O.** 2013. European Ash (*Fraxinus excelsior*) Dieback – A Conservation Biology Challenge. *Biological Conservation* 158: 37–49.
- Pliura, A., Lygis, V., Suchockas, Y. and Barteviceus, 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.
- Rosenvald, R., Drenkhan, R., Riit, T. and Lõhmus, A.** 2015. Towards Silvicultural Mitigation of the European Ash (*Fraxinus excelsior*) Dieback: The Importance of Acclimated Trees in Retention Forestry. *Canadian Journal of Forest Research* 45: 1206–14.
- Sevanto, S., Mcdowell, N.G., Dickman, L.T., Pangle, R. and Pockman, W.T.**, 2014. How Do Trees Die? A Test of the Hydraulic Failure and Carbon Starvation Hypotheses *Plant, Cell & Environment* 37: 153–61.
- Skovsgaard, J.P., Thomsen, I.M. Skovgaard, I.M. and Martinnussen, T.** 2010. Associations among Symptoms of Dieback in Even-Aged Stands of Ash (*Fraxinus excelsior* L.). *Forest Pathology* 40: 7–18.