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Ash dieback in Flanders (Belgium): research on disease development, resistance and management options

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Introduction

In the last two decades, the vitality of the European common ash, *Fraxinus excelsior* L., has rapidly deteriorated in northern, eastern and central Europe. The disease is caused by the invasive pathogenic fungus *Hymenoscyphus fraxineus*, which causes severe symptoms and dieback in common ash. In the Flemish region, common ash is a species which often grows in mixed stands together with other broadleaved species. The first official records of *Hymenoscyphus fraxineus* in Flanders date back to 2010. In this paper we present preliminary results of a study, investigating the condition of ash and the distribution and the extent of ash dieback. Subsequently, ongoing research into breeding and selection for resistance to *Hymenoscyphus fraxineus* is discussed. Finally management options applied to mitigate the impact of ash dieback are reported.

Importance of ash in the Flemish region of Belgium

In the Flemish region, common ash is growing mostly in mixed stands of high forest, coppice, or coppice with standards. Even though it is not the most common tree species, ash is well represented in many broadleaved forests. These forests are often situated in special protected areas. Ash is also an important non-woodland tree and it has been frequently used in urban areas. Ash disposes of a high ecologically and economically value as well. Throughout many centuries, the tree has been used not only for timber but also for tools and firewood. It has been planted in forests, parks, gardens, hedges and tree rows. Ash is also known as a pollard tree and in earlier days, these trees provided fodder for livestock (Pautasso et al., 2013). Moreover, ash is well-known for its high quality litter. The leaves are decomposing fast and generate good quality humus. Ash grows in forests with a high biodiversity. In the UK, 953 species were identified as associated with *F. excelsior* (Mitchell et al., 2014). The growth of ash reaches its optimum on loamy soils whereas it is almost absent on poor sandy soils. In Flanders, the autochthonous provenances can be found in the Loam region (Maes et al., 2006) as well as in the Polders region. The latter region is also an important station for ash, proving that ash is well growing on clayey soils.

The species is present in several habitats from the European Habitats Directive (Decleer, 2007), of which the most important one is habitat 91E0. This is a group of wet forests typically on water saturated sites and along streams, rivers or springs. They are known as *Alno-Padion, Alnion incanae* and *Salicion albae* and are divided in two groups: ash-alder forests (group D) and elm-ash forests (group E). The area of forest belonging to group D is estimated 31000 ha and for group E circa 800 ha. The habitat 91F0 is extremely rare (about 10 ha) and its distribution in Flanders is limited to the Meuse river. It is a typically mixed forest with *Quercus robur, Ulmus laevis, Fraxinus excelsior* or *Fraxinus angustifolia* (*Ulmenion minoris*). Ash is however not restricted to forest types 91E0 and 91F0. It is also a characteristic species in beech and oak forest habitats like type 9130, 9150 and 9160.

The first Flemish Forest Inventory was carried out by the Agency for Nature and Forests in 1997-1999. According to this inventory 63.6 % of the forest consists of homogeneous stands (one species > 80 % of the basal area). Only 0.2 % of the homogeneous stands are *Fraxinus excelsior* forests (Waterinckx & Roelandt, 2001). Ash occurs mainly in mixture with other tree species, either individually or in small groups. The species represents 2.5 % of the growing stock in broadleaved forest in Flanders, totalling 428000 m^3. Based on the calculated growing stock of both conifer and broadleaved forest, ash accounts for 1.4 %. As for basal area, ash is the eighth most important broadleaved species for growing stock. Regeneration of ash is found on 3.3 % of the inventory area.

Ash dieback and its spread (disease history)

The first official records of *Hymenoscyphus fraxineus* in the Flemish Region date back to 2010, when the infection was found in 2 stands in the provinces of Oost-Vlaanderen (municipality of Schorisse) and Vlaams-Brabant (municipality of Liedekerke). The pathogen could be isolated on young ash trees in both locations and its identity was confirmed by PCR (tests by A. Chandelier). However, the analysis of stem disks, taken at the necrotic lesions on the stem of a diseased tree collected at Liedekerke, revealed that the infection was already present on this tree in 2007. In 2011 a technical brochure with information on symptoms, ecology and management options of ash dieback was compiled (Roskams & De Haeck, 2011) and an information campaign on ash dieback was launched. At the same time a questionnaire was circulated among forest and green managers, aiming at a first state-of-the-art regarding the presence of the disease in Flanders. Their reports were checked through field visits by experts and through analyzing photographs of relevant symptoms. The presence of the disease was confirmed in 49 locations, spread all over the Flemish Region (Roskams & De Haeck, 2011). Ash dieback was reported mainly in young trees, in particular in new plantations (1-5 years). The intensity of the damage was varying, with dead branches and lesions on the bark being the more common symptoms reported. However in 40 % of the reports, also dead trees were mentioned. They all concerned young ash trees (< 20 years), but in most stands the number of dead trees was quite low (≤ 10 %). Only a few reports mentioned considerable losses of > 50 % of dead trees in young plantations. Additional information on the presence of ash dieback in Flanders was available through the yearly forest condition survey, based on a systematic 4 x 4 km grid (Sioen et al., 2015).

Research regarding ash dieback

Condition of ash in Flanders

In 2014 a long term project on ash dieback was started, aiming at the conservation of common ash as an important tree species in Flanders. The goal of this project is to describe the condition of ash, to investigate the distribution and the extent of the damage due to ash dieback and the selection of autochthonous ash trees, tolerant to ash dieback. At the start of the project ash dieback was known to be widespread mainly in young plantations (Roskams & De Haeck, 2011). Information on the condition of older ash trees in forest stands however was lacking. Therefore, in 2014 and 2015, the survey focused on the older trees. Field plots were selected from the Flemish Forest Inventory, the Flemish Forest Condition Network and other projects. The majority of these plots was situated on loam (37 %) and sandy loam (28 %) soils. In total we assessed 76 plots of which 29 have been sampled both in 2014 and 2015 (Fig. 1). In each plot a minimum of 3 and up to 20 ash trees were selected. We restricted the observations to dominant and co-dominant trees. In total we assessed 572 trees, of which 252 were observed in both years. If at least one infected tree was observed in a plot, the plot was scored positive for presence of *Hymenoscyphus fraxineus*. Simultaneously, site characteristics and soil type (texture and moisture class) of the plots were described in order to investigate their impact on the extent and the development of the disease symptoms.

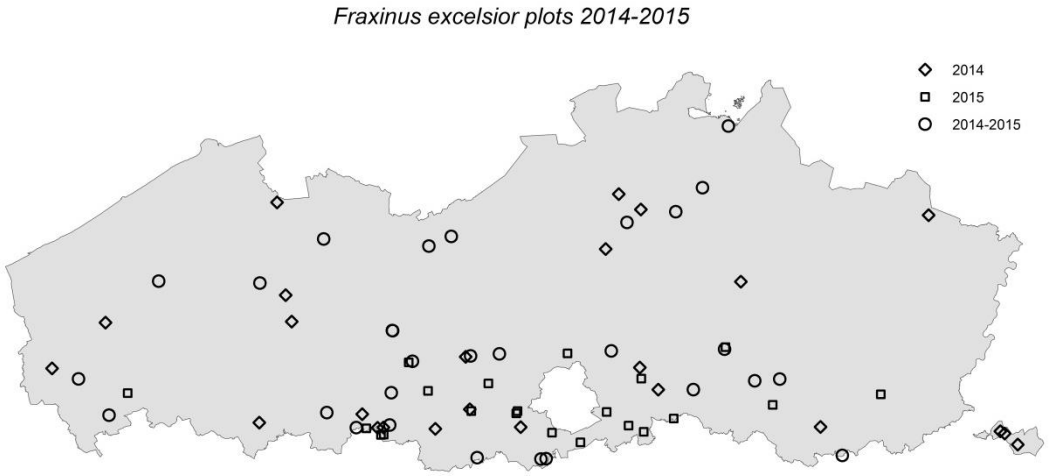

Figure 1 Monitoring plots in Flanders (2014-2015)

Surveys were conducted between June and September. The condition of the trees was assessed focusing on crown defoliation, crown dieback and other symptoms on the leaves, the branches and the stem, including biotic and abiotic damage factors. Defoliation was visually assessed in 5 % classes. Special attention was paid by the observers to the occurrence and the extent of symptoms, which may be due to *Hymenoscyphus fraxineus*, including discoloration of leaves and wilting of shoots (Fig. 2), bark necrosis, elongated cankers on stem and branches and dieback of upper parts of the crown. The presence of the characteristic, bushy appearance of tree tops as a result of repeated or alternating dieback and regrowth was also recorded.

Figure 2 Wilting of shoots and necrotic lesions due to *Hymenoscyphus fraxineus* (A. De Haeck)

Additional data collected on all trees were diameter at 1.3 m, information on fruiting and the occurrence of epicormic shoots. For these assessments we applied the methodology described in the ICP Forests Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests (Eichhorn, Roskams et al., 2010). Finally in each plot the occurrence of natural regeneration and the presence of ash dieback on these young trees was assessed.

First results show that in 2014-2015, symptoms of *Hymenoscyphus fraxineus* infection could be observed in 74 out of 76 plots (97 %). On tree level, symptoms of the infection were detected on 90 % of the sample trees. Within one year, the share of the common sample trees not infected by *Hymenoscyphus fraxineus*, decreased from 19.1 % in 2014 to 9.1 % in 2015. These results prove that the pathogen is now widespread in Flanders, not only on young trees in plantations or natural regeneration, but also on older trees in forest stands.

The distribution of the sample trees over 5 % defoliation classes (Fig. 3) indicates that the crowns of many trees are in a poor condition: 32.2 % of the trees show moderate defoliation (26-60 %), 11.0 % show severe defoliation (61-99 %) and 1.9 % of the trees are dead (Table 1). In comparison to other tree species in the Flemish region, common ash actually shows the highest average defoliation (34.8 %) and the highest share of moderately and severely defoliated trees (Table 1).

The survey results confirm that crown dieback, in various stages, is one of the most commonly observed symptoms. Only a very small portion of the trees (1.6 %) show dieback of current year shoots only, while bigger branches are unaffected. This group of trees has low defoliation scores, indicating that these trees have been infected only recently or – alternatively – they may show some tolerance to the disease. Trees with dieback of

current year shoots and small branches (up to 2 cm diameter) are more numerous and represent 7 % of the sample trees. The majority of the trees (62 %) show dieback of bigger branches (> 2 cm).

Genetics of ash / population and resistance

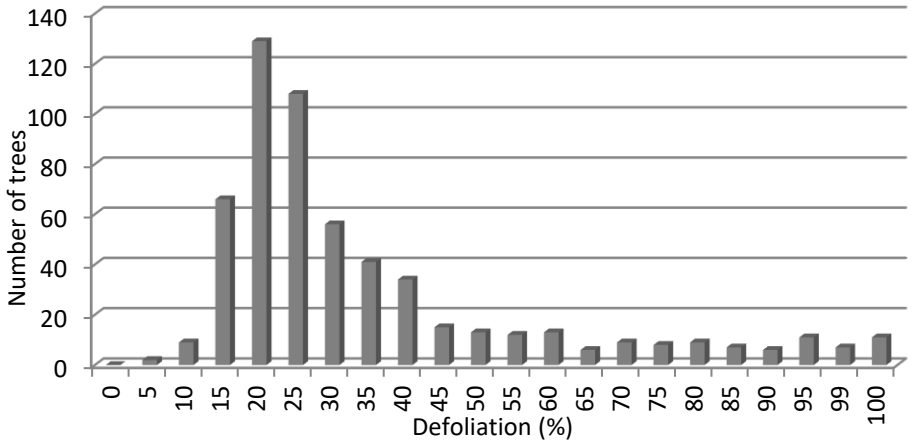

Figure 3 Defoliation of the sample trees in 5 % classes in 2014-2015.

Important evidence for the existence of a strong genetic component in resistance of *F. excelsior* to ash dieback has been proved from the analysis of existing field trials in affected areas under natural infections in several European countries. Pliura et al. (2011) and Kjær et al. (2012) also estimated the levels of additive variation and narrow-sense heritability in the degree of resistance of *F. excelsior* to ash dieback. Both studies confirmed the presence of additive genetic variation, in support of the ability of less susceptible parents to pass this trait on to their offspring.

Table 1 Distribution over defoliation classes (% of sample trees) and average defoliation (%) of broadleaves in the Flemish region (forest condition survey 2015; common ash: results 2014-2015).

Species	Defoliation class				Average defoliation
	0 - 25 %	> 25 - 60 %	> 60 - 99 %	100%	(%)
Common ash (*Fraxinus excelsior*)	54,9	32,2	11,0	1,9	34,8
Common oak (*Quercus robur*)	76,2	22,0	0,5	1,3	24,0
Beech (*Fagus sylvatica*)	90,7	8,5	0,0	0,8	19,7
Broadleaves	77,2	17,6	3,5	1,7	25,2

A first research project on genetics of resistance to ash dieback was started in 2014. In 1992-1993, 39 *Fraxinus excelsior* clones were phenotypically selected as plus-trees in natural mixed stands in the Flemish region, i.e. healthy trees showing good stem form and vigour. In addition, all clones were selected for resistance to bacterial canker by artificial infection with an aggressive strain of *Pseudomonas savastanoi pv. savastanoi* on 10 ramets/clone (results not yet published). Plus-trees were selected well before ash dieback was observed. Scions of each plus-tree were grafted onto root stocks and planted in a seed-orchard in 2006. When ash dieback symptoms became visible in the orchard in 2013, resistance to ash dieback disease was yearly analyzed by classifying the damage sustained to the crown and the stem. In 2015, after 2 years of observation, only a very limited number of the 39 plus-trees showed reduced susceptibility. Selection of tolerant trees will be finished in 2016. A second project is going on in Flanders to study the genetic component of resistance and to select interesting disease tolerant genotypes in a European provenance trial installed at two sites in 2005. The trials include 50 provenances from 10 European countries with 108 trees per provenance. Ash dieback symptoms and phenology, among other traits, will be assessed on all trees. Seeds will then be collected on the disease tolerant genotypes aiming at the estimation of narrow-sense heritability. Genotypes tolerant to *Hymenoscyphus fraxineus* will be vegetatively propagated and screened for resistance to *Pseudomonas savastanoi pv. savastanoi* by artificial infection. Only disease tolerant ash genotypes will be included in a new seed orchard.

Impact of ash dieback on cultivation and planting of ash

Planting ash has been promoted for a long time by means of an important subsidy from the Flemish authority. Statistics from the Agency for Nature and Forests show that between 2003 and 2014 around 14% of the subsidized area for afforestation or reafforestation concerned ash. Most of these plantations are now suffering from infection by *Hymenoscyphus fraxineus* or died and were replanted. After 2011 the yearly afforested area with ash decreased remarkably. In 2016 a subsidy is still granted for planting *Fraxinus excelsior*, but only when ash is mixed with other species. The cultivation of planting material is an important business for several tree nurseries in Flanders. The Flemish Department of Agriculture and Fisheries is responsible for the inspection and certification of planting material, following the EU Forest Reproductive Materials Directive. The sales figures for *Fraxinus excelsior* decreased from 879541 plants in winter 2009/2010 to 23531 in 2014/2015 (Fig. 4). About 10 nurseries in Belgium are still cultivating and exporting planting material of ash to other EU-countries.

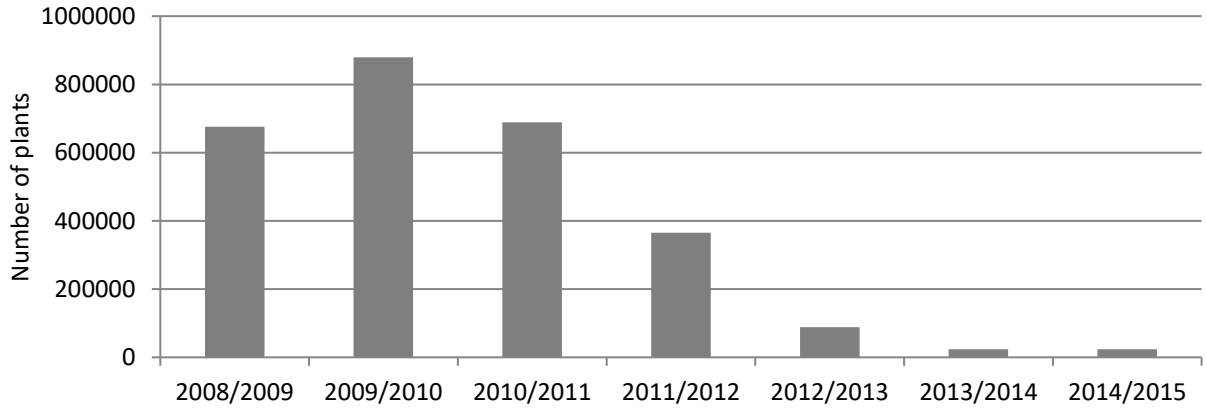

Figure 4 Trade of certified planting material of *Fraxinus excelsior* in Flanders.

Management options to mitigate the impact of ash dieback

Silvicultural management options: Recommendations for forest management

In 2011 a report on the status of ash dieback in Flanders was published, including practical advice for forest managers (Roskams & De Haeck, 2011). In 2013, additional recommendations for forest management were provided at the request of the Flemish Agency for Nature and Forests (De Haeck et al., 2013). A summary of the recommendations is given below.

Inspection of ash stands should be done preferably during summer, since this is a good time to assess foliar discoloration, wilting shoots and dead branches. Healthy-looking ash trees should always be preserved. Foresters don't have to cut all the trees that are lightly or moderately damaged because some of them may be tolerant to ash dieback. Heavily infected trees with a high risk of decreasing timber value should be cut without delay if the wood is to be sold on the market. Declining trees may be cut as well as trees with epicormic branches after *Hymenoscyphus fraxineus* -infection. Reason for this is that, when epicormic shoots die, the infection reaches the wood and causes wood discoloration. Discoloured wood partly loses its market value.

In severely infected young stands with a high mortality and no natural regeneration, it is recommended to cut ash and to replant with other species. (Re-)planting ash on large areas is not recommended because of the high infection risk and as long as no disease tolerant ash is on the market. If a forest owner nevertheless wants to plant ash, small groups are recommended, mixed with other species. On diseased trees, it makes no sense to cut infected shoots, twigs or branches. Thinning operations however, should favour other species and reduce competition. Suitable alternative species could be introduced after cuttings. Dead trees do not play a role in the development of the disease and so for biodiversity reasons, dead trees and trees close to death should be left in the forest. Open spaces with dead trees could enable natural generation.

Ash trees that have died recently may be hosting ash bark beetles (*Leperisinus varius*). Once the bark is peeling off, dead trees are no longer susceptible to bark beetle infestation. However, declining trees are also susceptible

to weakness parasites. Honey fungus (*Armillaria spp*.) is such a secondary pathogen, frequently associated with ash dieback and able to kill a tree. From an economic point of view, *Armillaria* - infected trees may be cut. But cutting these trees is not an essential mean to reduce the spread of *Hymenoscyphus fraxineus*.

People should be informed about safety risks. Dead branches and declining parts of the crown may pose a risk, especially for forest workers. A detailed and frequent inspection of ash trees along roads is recommended. Dead and dying trees along forest roads or footpaths should be cut.

Alternative tree species

The choice of alternative tree species depends on different factors, for instance site conditions. It is recommended to maximise the use of species growing together with ash under natural circumstances. Besides species like oak (*Q. robur*), alder (*Alnus glutinosa*) and sycamore (*Acer pseudoplatanus*) other suitable species include *Populus sp., Salix sp., Ulmus sp., Tilia sp., Prunus avium, Carpinus betulus, Castanea sativa, Fagus sylvatica...*

Conclusions

The first official records of *Hymenoscyphus fraxineus* -infection in Flanders date back to 2010, but first symptoms of the disease has been shown to be present already in 2007. At that time, ash dieback was mainly reported on young trees and in new plantations. In 2011, an information campaign on ash dieback was launched, including a technical brochure with information on symptoms of the disease and preliminary management guidelines. A survey in 2014 and 2015 showed that the crown condition of ash is currently also deteriorating in older stands, but the mortality rate is still lower compared to younger plantations. Symptoms of infection by *Hymenoscyphus fraxineus* have now been observed all over the Flemish region of Belgium.

Despite the spread and the intensity of the disease, some nurseries in Flanders keep on producing ash trees and the species is still planted in and outside the forest. Afforestation or reafforestation with ash is still subsidized by the Flemish government, but it is recommended to use the species only in mixed plantations. Since 2011, cultivation and selling of ash planting material has decreased whereas cutting of ash will probably increase.

Preliminary results of resistance screening in a seed orchard of 39 autochthonous plus trees indicate that only a very limited number of plus trees will be tolerant to ash dieback. Therefore, a new selection of plus-trees is started, so that in the near future, nurseries can hopefully be provided again with seeds from disease tolerant, autochthonous trees. Breeding and selection for tolerance of ash trees in a European provenance trial will be continued. This is essential, as large effective population sizes will be required to avoid genetic bottlenecks.

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