

Spread of Ash Dieback in Norway

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Solheim, H. and Hietala, A.M. 2017. Spread of Ash Dieback in Norway. *Baltic Forestry* 23(1): 144-149.

Abstract

Ash dieback, caused by the ascomycete *Hymenoscyphus fraxineus*, was first observed in the eastern and southernmost Norway in 2008. Based on the age of stem bark lesions, it was concluded that the fungus had arrived to the region no later than 2006. Since 2008 the annual spread of the disease northwards along the west coast of Norway has been monitored. The registration was done each year during early summer around a disease frontier recorded in the previous year. The occurrence of necrotic bark lesions in the previous-year shoots and dieback of these shoots, and isolation of *H. fraxineus* from the discoloured wood associated with necrotic bark lesions were used as signs of ash dieback. These records indicate an annual spread of ash dieback in the range between 25 km and 78 km, and a mean annual spread of 51 km. The cause of the spread is discussed.

Keywords: Ash dieback, *Hymenoscyphus fraxineus*, monitoring disease spread, west coast Norway

Introduction

Ash dieback was first observed in Poland in the early 1990ies (Przybył 2002). The disease spread first to the neighbour countries such as Lithuania and has later reached most stands in Europe where common ash (*Fraxinus excelsior* L.) is growing (Timmermann et al. 2011, McKinney et al. 2014). The cause of ash dieback was for a long period unknown, but in 2006 the anamorphic fungus *Chalara fraxinea* T. Kowalski was described as the cause (Kowalski 2006). The teleomorph was assigned to genus *Hymenoscyphus*, and the species was first named *H. pseudoalbidus* (Queloz et al. 2011). The new codex rules adopted at the International Botanical Congress in 2011 advocated the abandoning of dual naming system for pleomorphic fungi. As a result, the oldest epithet *C. fraxinea* was combined with the teleomorph genus and the species was renamed as *H. fraxineus* (T. Kowalski) Baral, Queloz & Hosoya (Baral et al. 2014).

The ash dieback pathogen can spread to new geographic regions via several pathways. One is trade or movement of various material infested by the fungus (Sansford 2013, McKinney et al. 2014). Seedborne spread (Cleary et al. 2013, Drenkhan et al. 2016) and transfer and planting of seedlings infected in the nursery conditions provide spread pathways (Schumacher et al. 2010, Kirisits et al. 2012) that may have facilitated the disease spread to UK and Ireland (Sansford 2013). Even timber transport may be a source of spread of ash dieback, since the fungus is able to establish infection in bark and wood at stem collar

in large trees (Husson et al. 2012, Chandelier et al. 2016, Marçais et al. 2016). Airborne spores provide a pathway for natural spread of most fungi, but the effective distance of this route depends on many factors (Ingold 1971). An example of fungi with long distance spread of spores is species in genus *Heterobasidion*, whose basidiospores have been sampled more than hundred kilometres away from the nearest possible source (Rishbeth 1959, Kallio 1970). During the sporulation season, ascospores of *H. fraxineus* are simultaneously liberated in vast amounts in the early morning, presumably due to active discharge. The maximum sporulation of *H. fraxineus* occurs in July and August (Hietala et al. 2013, Chandelier et al. 2014, Dvorak et al. 2016). Their amount in air is drastically reduced already a few hundred meters away from a diseased ash stand (Chandelier et al. 2014, Steinböck 2013). Since the first European recording of ash dieback in Poland around 1992, most of the common ash forests in Europe have become infested by *Hymenoscyphus fraxineus* (Figure 1).

In Norway, ash dieback was first recognized in 2008 (Talgø et al. 2009) in a plant nursery which had imported ash plants from Sweden. The nursery manager had observed shoot dieback on ash seedlings already in 2007, but thought, as many others, that it was damage caused by winter frost, since these symptoms were present in a large area covering a distance of more than 350 km measured on the map. However, when searching for disease symptoms in 2008, also bark necroses where the vascular cambium had died already in 2007 were found, and it was concluded that the first infections had taken place already in 2006 (Sol-

heim 2012). The current paper describes the spread of ash dieback in Norway in the period between 2008 and 2016 along the west coast in Norway.

Material and methods

Every year after leafing, at turn of May- June or later in the summer, ash trees up to 8-10 meters height were inspected for shoot dieback and presence of bark lesions: the survey was started at the place where the disease front had been observed the previous year, and extended into the region considered as healthy the previous year until no signs of ash dieback were observed. A few times the previous year front had to be redefined due to the discovery of old bark lesions in an area considered as healthy the previous year. The distance between an old and a new front was measured as a straight line on the map.

Shoots with shoot dieback or stem bark lesions, sampled at the new front, were brought to the laboratory for fungal isolation. The shoots were split and small tissue pieces taken at the edges of discoloured wood were placed on malt extract agar (MEA, 1.5% malt and 1.5% agar) in Petri dishes. The Petri dishes were inspected regularly for the presence of phialophores characteristic to the vegetative phase of *H. fraxineus* (Kowalski 2006). Representative isolates were later grown on a cellophane membrane placed on malt agar. The harvested mycelia were ground in liquid nitrogen using a mortar and subjected to DNA isolation with Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR amplification was carried out with ITS1 and ITS4 primers (Gardes and Bruns 1993) in 50- μ l-reaction volumes using HotStarTaq™ Plus DNA Polymerase (Qiagen) according to the manufacturer's instructions. After gel electrophoresis, the amplicon from each reaction was purified with MinElute PCR purification Kit (Qiagen) and sequenced in both directions on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Contigs were assembled with Seqman software (Lasergene, DNASTAR Inc.,

Madison, WI) and queried against ITS sequences in NCBI GenBank Sequence Database.

Results

During the first search in 2008, symptoms characteristic to ash dieback were recognized in a huge area that extended 360 km south from Hedmark county (Ringsaker municipality, Nerlia 60.83494423°N, 10.91020265°E). The south-easternmost finding was ca. 1 km in Rogaland county (Lund municipality, Skår, 58.44918°N, 6.56468°E) (Table 1, Figure 2). The site in Rogaland was an open area near the main road, where a few diseased ash saplings, obviously naturally regenerated, were observed. The nearest forest habitat with ash dieback was located along a small river near the fjord close to Flekkefjord town in Vest-Agder county.

In 2009 the front of ash dieback was observed in Eigersund municipality (Litle Hogstad) in Rogaland county ca. 25.5 km northwest of the previous year's front (Table 1, Figure 2B). At this site a few diseased ash saplings were growing along a river in an open landscape with agricultural fields on both sides of the river. A young ash stand with few diseased saplings was located about 1.5 km east of the front.

In 2010 the new front was found in Gjesdal municipality in Rogaland (Ålgård) (Table 1, Figure 2B), 29.5 km away from the previous year's front. Here a few ash saplings showed stem lesions or shoot dieback in two places in the village and a few locations outside the village around the lakes Edlandsvatnet and Limavatnet.

In 2011 a few diseased saplings were observed just north of the front of 2010, but further north near Jørpeland (59.0170353N, 6.04809061E) also two-year-old bark lesions were observed in ash saplings. At Hogganvika in Vindafjord municipality (59.47992973°N, 5.92234053°E) even older necroses were found and especially one site showed dead small ash trees and saplings with symptoms

Table 1. Spread of ash dieback at the west coast of Norway with information about the year observed, county, municipality, site, and coordinates of the deduced diseased fronts. The column distance indicates the distance of the deduced disease front site to the disease front observed the previous year

Year	County	Municipality	Site	North°	East°	Distance (km)
2008	Rogaland	Lund	Moi	58.44913233	6.56457068	
2009	Rogaland	Eigersund	Litle Hogstad	58.54115054	6.16421514	25.5
2010	Rogaland	Gjesdal	Ålgård	58.7692211	5.91129864	29.5
2011	Sogn og Fjordane	Askvoll	Rivedal	61.36118896	5.24321179	(52.5-70)
2012	Sogn og Fjordane	Flora	Sunnarvåg	61.65853028	4.99647677	36
2013	Møre og Romsdal	Herøy	Nykreim	62.25528828	5.79300963	78
2014	Møre og Romsdal	Ørskog	Sjøholt	62.55258389	7.67142575	59
2015	Møre og Romsdal	Averøy	Bruhagen	63.05479556	7.63431558	76
2016	Møre og Romsdal	Aure	Våg	63.28595052	8.5410134	53
Mean 2008-16						51



Figure 1. Map of Europe showing the distribution range of *Fraxinus excelsior* (in blue) and the year of the first observation of symptoms of ash dieback or the year it was supposed to have arrived in the country for the first time. Most information is based on references. In addition Dr. R. Vasaitis and Dr. N. Keca have contributed with personal information from some countries. The distribution map of common ash was kindly provided by EUFORGEN (2009)

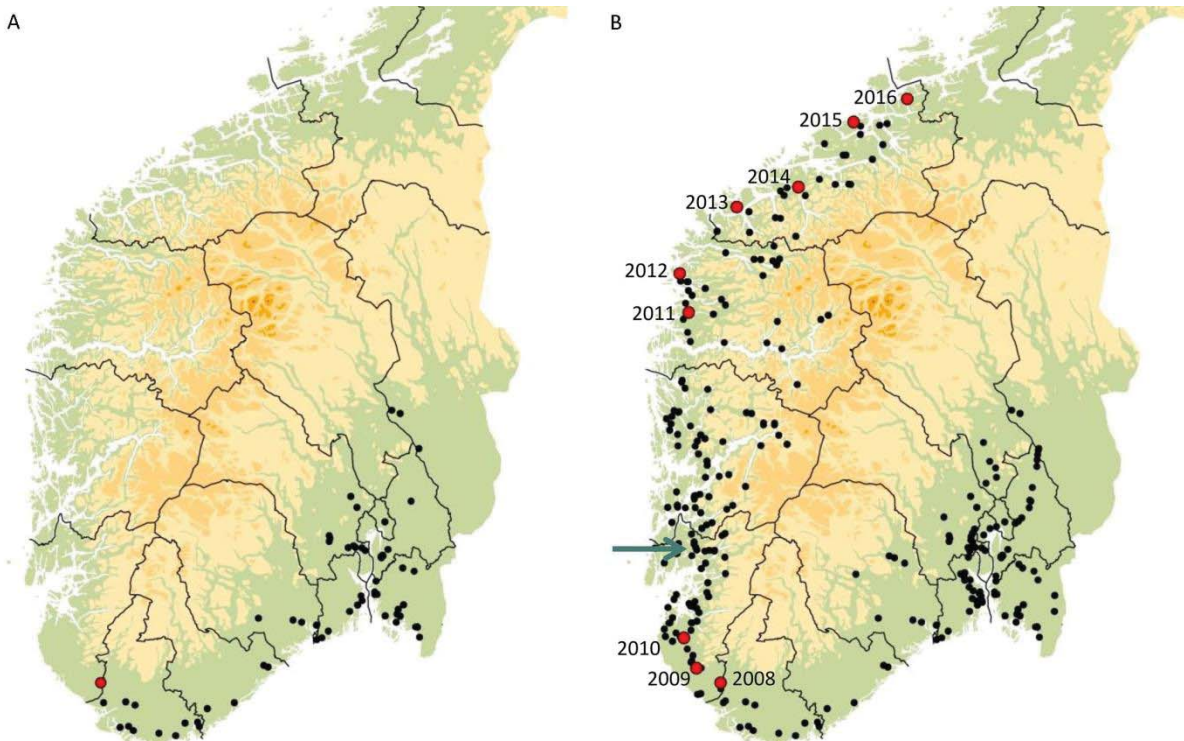


Figure 2. A. The distribution of ash dieback in Norway in 2008. All dots are verified with isolations and many also with sequencing. B. The known distribution of ash dieback in Norway in 2016. Red dots are front sites each year at the west coast. Green arrow shows the area in Vindafjord municipality (Hogganvika), where a separate introduction is supposed.

that were concluded to be 3-4 years old (Figure 2B, big arrow). Even further north, one-year-old necroses were found up to Askøy, just outside Bergen.

So in 2011 the new disease front had reached Sogn og Fjordane county, and thereby passed Sognefjorden, the longest fjord in Norway. The front was in Askvoll municipality (Rivedal) (Table 1, Figure 2B). Here the fjord Dalsfjorden was surrounded by small patches of agricultural fields, and small ash trees were found scattered in the area. Only one stem bark lesion was found here. The distance of the site to Hogganvika is 210 km. Since it most probably has been an own introduction to Haugalandet, the distance of spread in 2011 could not be estimated. If the spread from Hogganvika had taken for example 3 or 4 years, the annual spread had been 70 km or 52.5 km, respectively.

In 2012, the disease front site in Sogn og Fjordane was just south of Sunnarvåg (Table 1, Figure 2B) in Flora municipality in a mixed broadleaved stand with ash saplings and small plants growing in a cove a few meters from the Atlantic Ocean. This stand was first recognized in 2013, but some of the lesions observed then were concluded to be one year old. The distance of this site to the previous year's front in Rivedal, Askvoll is ca. 36 km (Table 1, Figure 2B).

In 2013 the disease front was observed in Herøy municipality (Nykreim) in the county of Møre og Romsdal (Table 1, Figure 2B). The site hosted ash saplings and small trees, two of which had symptoms of ash dieback. The distance to the 2012 front at the coastline is 78 km.

In 2014 the disease front in the coastal region of the Møre og Romsdal county was at Sjøholt in Ørskog municipality (Table 1, Figure 2B), locating 59 km north of the 2013 disease front. Here there were as many as 20 ash trees with one or several stem bark lesions or shoot dieback, and this was the most severely affected site at the front any year.

In 2015 the northernmost site with diseased ash along the coast was in Averøy municipality near Bruhagen (Table 1, Figure 2B), locating 76 km north of the previous year's disease front. The site hosted many big and small ash trees in an agriculture area.

In 2016 the northernmost finding of ash dieback symptoms was near Våg in Aure municipality (Table 1, Figure 2B), 53 km north of the previous year's disease front. Here one of the small ash trees growing along a small river (Vågselva) had a necrosis on the main stem.

Based on vegetative morphology, a total of 106 isolates obtained from symptomatic tissue of common ash were identified as *H. fraxineus* (Table 2). Out of the 34 *H. fraxineus* isolates subjected to molecular identification by ITS rDNA sequencing, 32 showed 100% sequence similarity to the holotype of *Chalara fraxinea* (FJ597975), while

one of the remaining isolates showed a one-nucleotide-insert and the other a point mutation within the ITS1 region.

Table 2. Number of *Hymenoscyphus fraxineus* obtained from annual disease fronts and the subset subjected to ITS rDNA sequencing

Year	Isolates obtained	Isolates sequenced
2009	12	5
2010	9	5
2011	51	16
2012	4	0
2013	20	5
2014	9	3
2015	0	0
2016	0	0

Discussion

The survey for the occurrence of ash dieback was done annually during early summer. The examined stem bark lesions were deduced to be < 1 year old if the lesion area and adjacent healthy tissue showed the same number of year rings. In such cases the infection was concluded to originate from previous year, since the sporulation period of *H. fraxineus* in Norway extends from the end of June to October, and the peak sporulation occurs in the period between mid-July – mid-August (Hietala et al. 2013). The dieback of shoots is easy to see, but this may be caused also by some other fungi and by winter frost damage as well. To be sure that shoot dieback or a stem lesion was caused by *H. fraxineus*, tissue samples taken at a deduced disease front were always brought to the laboratory for fungal isolations. The identification of *H. fraxineus* was based on the typical anamorph characters of the species (Kowalski 2006), and in addition the identity of many of the isolates was confirmed by sequencing of the ITS rDNA gene cluster.

To search for ash dieback over a huge area is complicated, especially in the west coast of Norway that is shaped by fjords and mountains and is often poorly accessible by roads. Therefore there is obviously uncertainty whether the exact front of ash dieback was recognized every year, and indeed sometimes older necroses were found up to a few kilometres north from the disease front deduced during previous year's survey. In such a case the front was "moved" to the new site. Generally, the recognized front was probably not far from the real front. The distances of the estimated annual disease spread are thus not exact, but rather approximate.

The rapid spread of ash dieback through Europe is still not fully understood, but many factors may have contributed, including trade and movement of infected seeds (Cleary et al. 2013), ash seedlings and other plant material

(Schumacher et al. 2010, Kirisits et al. 2012, Gross and Holdenrieder 2013, Sansford 2013), timber (Husson et al. 2012), and natural spread of windborne ascospores over unknown, but probably substantial dispersal distances. The first observation of the pathogen in the UK in 2012 was made on seedlings imported from the continent. However, subsequent spatial mapping of infected trees in natural stands implies that *H. fraxineus* spread to the UK by wind-dispersed spores crossing the North Sea from continental Europe, infecting natural stands particularly in the south-eastern and eastern parts of the country (Sansford 2013, www.ashtag.org).

Regarding Norway, in 2008 the Norwegian Food Safety Authority laid down regulations with the aim to prevent the spread of ash dieback to areas in Norway deemed to be free of infection. These regulations divided the country into a quarantine zone (southeastern counties), an observation zone (Rogaland county) and an infection-free zone (the rest of the country). These regulations concern ash plants, propagating material and wood, and prohibit import of such material from the quarantine zone into observation and infection-free zones. Moreover, it must be officially verified that no symptoms of ash dieback have been observed on the plant material to be placed on the market in the infection-free and observation zones (<https://lovdata.no/dokument/SF/forskrift/2008-09-08-1005?q=FOR-2008-09-08-1005>). After detection of ash dieback in 2008 in Norway (Talgø et al. 2009), there was extensive publicity around this disease that reduced greatly the demand for ash seedlings for planting. Nurseries with ash seedling production destroyed ash plants to give space to saleable plants. An exception was a nursery located in Trøndelag far away from the diseased area. Ash dieback has not reached Trøndelag even in 2016, and this nursery is still selling ash plants locally, but very few (Wormdal nursery, pers. comm.). Because of regulations and stopping of commercial ash seedling production, it is likely that the spread of ash dieback recorded now in the west coast relates to airborne spread of the fungus or alternatively, originates from previous transfer of infected plant material.

Our records indicate an annual spread of ash dieback in the range from 25 km to 78 km, with a mean of 51 km each year. These estimates are well in line with the annual spread of 75 km estimated for *H. fraxineus* in Europe (Gross et al. 2014). A similar spread distance has also been observed in northern Italy (Luchi et al. 2012). They reported a spread of ca 50-60 km each year westward near the border to Slovenia.

The annual 75 km disease spread estimated by Gross et al. (2014) is the total spread which could have been facilitated by movement of plants from nurseries as seen in UK and Ireland (Sansford 2013). Trade-facilitated spread was probably more important in the beginning of the disease spread, since the cause of ash dieback by *Chalara fraxinea*

was first described in 2006 (Kowalski 2006). At that time the disease had been in Europe for more than 10 years (Przybył 2002) with free movement of ash plant material.

Steinböck (2013) showed that the ascospore amount of *H. fraxineus* in air reaches a low plateau already by 160 m away from an infested stand. Similar results were obtained by Chandelier et al. (2014) who recorded a comparable background level of pathogen ascospores 50 to 500 m away from an infested stand when the wind speed was 20 km/h (5.6 m/s). In line with the now recorded spread of ash dieback along the west coast of Norway, the prevailing wind direction in this region is from south or south-west and the average wind speed during the pathogen sporulation season in July and August may be at a similar level (20 km/h). The effective dispersal distance the ascospores of *H. fraxineus* remain viable and the amount of ascospores required for shoot infection of common ash remains to be clarified.

Acknowledgements

Financial contribution has been given by the The Norwegian Genetic Resource Centre, The Research Council of Norway (grants 203822/E40 and 235947/E40), COST Action 1103 FRAXBACK and the Ministry of Agriculture and Food (131001). Laboratory work has been done by Gro Wollebæk, Anne Nilsen and Inger Heldal. Jørn Petter Storholt has made the maps.

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