Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Institutionen för skoglig mykologi och
växtpatologi

Dieback of European Ash (*Fraxinus* spp.)

– Consequences and Guidelines for Sustainable Management

Edited by Rimvydas Vasaitis & Rasmus Enderle

EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

Acknowledgement

EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

This publication is based on the work of Action FP1103 FRAXBACK, supported by COST (European Cooperation in Science and Technology)

COST (European Cooperation in Science and Technology) is a pan-European intergovernmental framework. Its mission is to enable breakthrough scientific and technological developments leading to new concepts and products, and thereby contribute to strengthening Europe’s research and innovation capacities.
It allows researchers, engineers and scholars to jointly develop their own ideas and take new initiatives across all fields of science and technology, while promoting multi- and interdisciplinary approaches. COST aims at fostering a better integration of less research-intensive countries to the knowledge hubs of the European Research Area. The COST Association, an international not-for-profit association under Belgian Law, integrates all management, governing and administrative functions necessary for the operation of the framework. The COST Association currently has 36 Member Countries. www.cost.eu

Dieback of European Ash (*Fraxinus* spp.)
- Consequences and Guidelines for Sustainable Management

The Report on European Cooperation in Science & Technology (COST)
Action FP1103 FRAXBACK

Edited by
Rimvys Vasaitis
Department of Forest Mycology & Plant Pathology
Swedish University of Agricultural Sciences, SE-75007 Uppsala, Sweden

and

Rasmus Enderle
Department of Forest Health, Forest Research Institute of Baden-Wuerttemberg, Freiburg, Baden-Württemberg, D-9100, Germany

Cover illustrations by
Indra Vasaitis

ISBN (print version) 978-91-576-8696-1
ISBN (electronic version) 978-91-576-8697-8

Print: SLU Service/Repro, Uppsala, 2017

COST is supported by the
EU Framework Programme
Horizon 2020

Fraxinus dieback in Europe: understanding disease phenomenon and elaborating guidelines for sustainable management (FRAXBACK)

(Abstract of FRAXBACK Action Proposal: July 28th, 2011)

Currently, severe dieback of *Fraxinus* spp. is observed in most European countries. This is an emerging disease, which results in massive tree mortality, threatening the existence of *Fraxinus* over the continent. It is caused by *Hymenoscyphus pseudoalbidus* (currently known as *Hymenoscyphus fraxineus*), alien and invasive fungus, origin of which remains unknown (nowadays known: Far East Asia). Currently, many European countries have national research programs on *Fraxinus* dieback, focusing on numerous aspects of the biology and ecology of the disease, but the activities are scattered. Aim of the FRAXBACK is, through sharing and synthesis of available knowledge, generate comprehensive understanding of *Fraxinus* dieback phenomenon, and to elaborate state of the art practical guidelines for sustainable management of *Fraxinus* in Europe. The Action will be implemented through innovative interdisciplinary approach, and will include forest pathologists, tree breeders and silviculturists. Its deliverables: i) guidelines for sustainable management of *Fraxinus* in Europe; ii) European database for dieback-resistant *Fraxinus* genotypes/families/populations and established/planned progeny trials; iii) illustrated digests/leaflets/brochures on *Fraxinus* dieback; iv) disease distribution maps; v) website; vi) book (*de facto* two books). FRAXBACK is comprised of four Working Groups: WG1 Pathogen; WG2 Host; WG3 Silviculture; WG4 Dissemination and knowledge gaps. Its duration is 4 years, including two MC/WG meetings and four STSMs per year (*de facto* a total of 37), and one international conference (*de facto* 10).

Dr Rimvys Vasaitis

Action Chair & GrantHolder

Foreword

European Cooperation in Science & Technology (COST) Actions provide excellent fora for promoting interactions between scientists involved in specialised research areas, mainly within the EU, but also including colleagues from all over the world. The FRAXBACK Action arose in response to the spread of ash dieback caused by an invasive fungal pathogen *Hymenoscyphus fraxineus*, which was probably accidentally introduced into northern Europe in the 1980s. The first outbreaks occurred in Northeastern Poland and in Lithuania in the 1990s, although the cause was unknown until several years later, following which the problem spread inexorably through much of northern Europe where common ash, *Fraxinus excelsior*, occurs and also into stands of narrow-leaved ash (*F. angustifolia*) further south in Europe.

With a disease threatening the future of yet another much loved native European tree, a number of research projects were initiated in northern Europe. The chapters in this book represent the majority of the work conducted within the COST Action and in the many associated projects carried out by partners in the consortium. Its publication is very timely, given the massive effort that has gone into determining the spread of the disease, alongside technological breakthroughs in genomics and metabolomics of the hosts and pathogen which have occurred in the last 3-4 years. Moreover, the book gives scientists new to the field a foundation on which to build new research into the problem of ash dieback and to the dynamics of invasion of alien fungal pathogens impacting on forest ecosystems.

Much of this research is summarized here; some is available in scientific journals, but the book itself represents the culmination of the huge effort made to understand ash dieback in Europe since the 1990s. The first three chapters focus on the implications of ash dieback disease in terms of the historical uses made of ash, and the impacts of this disease on society. The following chapter present overviews of the spread of ash dieback in Europe, along with other chapters addressing how we might address the issues arising from loss of ash.

The sister volume to this work, a special issue of *Baltic Forestry* on ash dieback, provides further detail on the work carried out within the framework of the COST Action, and should be read alongside this book.

Professor Steve Woodward

University of Aberdeen
Editor-in-chief, *Forest Pathology*

Contents

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 1 – 14.

Management and use of Ash in Britain from the prehistoric to the present: some implications for its preservation

J. PRATT

Cross House, Mountain Cross, West Linton, Peeblesshire, Scotland. EH46 7DF.
k.m.pratt@btinternet.com

Summary

The properties that make the wood of fast-grown Ash pliable, strong and resilient have been exploited by man for thousands of years, and are illustrated by reference to the probable use of Ash timber for tools, arms and transport by the Roman Army of occupation in Britain two thousand years ago. Militarily organised and disciplined, the Roman Army was responsible for changing the face of Britain with huge infrastructure projects that required significant numbers of tools, equipment and fuel, in addition to the arms it used to maintain control over the fractious tribes of the north. The extent to which it maintained supplies of this valuable resource by managing its woods, possibly by coppicing, is discussed and raises the question as to the degree of genetic selection involved in coppicing.

Background

Arguably, one of the most significant of man's achievements has been to work out how to fix a handle, or haft, to a stone and thus create an axe. This is a surprisingly difficult union to achieve using only stone tools, and early man exhibited great ingenuity in doing so, even to the extent of using pieces of antler as intermediate links between wood and stone (Maigrot 2011). The effect of the haft is to extend the radius of a man’s arm, thus trebling the length of arc of travel of the axe head and thereby increasing the speed of the stroke. By doubling the speed of travel of the axe head the force of impact is quadrupled (Mytting 2015). The provision of this significant mechanical advantage over hand-held axes enabled man to extend his lifestyle beyond that of a hunter-gatherer and manage his environment to provide a regular source of food and heat. Although stone axe heads are commonplace, few hafts have survived, and there is no clear date for this crucial invention. One exception is the Shulishader porcellanite stone axe from the Hebrides (Fig. 1 A, Fig.1 B) which has a haft tentatively identified as Rosaceae sp. The fact that this haft seems to have been reused suggests the value placed on a good handle. Ash, (*Fraxinus excelsior* L) which became the preferred timber to use in wooden hafts (Green 1978: Harding and Young 1979, Maigrot 2011), was uncommon in North Britain when this axe was made in the Neolithic: a C^{14} date of 3495-2910 BC (Sheridan 1992) for the handle fits with a known period of use of porcellanite stone axe heads imported from Northern Ireland (Cooney and Mandal 1998).

Although no wooden axe hafts have been found from the early Palaeolithic, there is clear evidence that by at least 60,000 years ago early humans already had a long history of making wooden handles and shafts for their stone tools, bound together with tree resins (Dinnis and Stringer 2014). These included shafts for spears made by Neanderthals 400,000 years ago (Thieme 1997). Although these shafts were of spruce, they provide precedence for all that followed. Later there is clear evidence (see below) that Ash had become the preferred species for spear shafts. What are the characteristics of Ash that make it so suitable for this purpose?

Ash timber properties

Ash is a ring-porous fast-growing native hardwood. It is easy to saw and machine when dry, and is the toughest of British grown woods (Brazier 1990). It has vasicentric axial parenchyma, i.e., a complete sheath of parenchyma cells around one or more vessels (Fig. 1 C). Over several years’ radial growth, the thickness of early wood remains fairly constant and any increase in ring width comes from the laying down of more late wood vessels, with a corresponding increase in density. Strength and toughness will therefore tend to increase with more rapid growth. Weaknesses in slow-grown Ash can be explained by the comparatively narrow layers of late wood that are laid down where short growing seasons restrict the amount of late wood formed. Variability of growth also depends on genetic or systematic effects: ring width tends to reduce with increasing age, so timber derived from old trees may be less dense and not as tough as that from young vigorous trees.

Figure 1 See next page for descriptions.

Figure 1 A) Neolithic Stone Axe, found at Shulishader, Lewis. The haft is probably Rosaceae spp. The head, from Ireland, is porcellanite. *The axe is in the National Museum of Scotland, Edinburgh and the photograph is reproduced with their permission.* **B)** Stone hand axes were man's only large tool for hundreds of thousands of years. **C)** Anatomy of *Fraxinus excelsior* (Ash). From left: cross (xs), radial longitudinal (rls) and radial tangential (tls) sections. Courtesy Peter Gasson, Kew. **D)** Coppiced Ash split from an ancient stool in Bradfield Woods and bent to form this chair, made by a local craftsman Dan Hussey, who kindly donated this photograph. Ash turns well (*Author*). **E)** Replica 1911 Bristol Boxkite, built by Miles Aircraft Ltd for the film "*Those Magnificent Men in their Flying Machines*" using authentic materials, which included Ash for the undercarriage skids. 6 May 1964, pilot George Miles FRAeS. *Photo courtesy Karen Miles.* **F)** Coppiced Ash stool, Bradfield Woods, Suffolk. Some of these stools are 800 years old and may have been cut at least 70 times. Note the high quality and vigour of these stems. **G)** The northern Frontier of Roman Britain. Aerial view east over the Roman fort of Housesteads, on Hadrian's wall. The curtain wall lies along the north wall of the fort and stretches to the horizon. The Vallum runs almost parallel to the Wall itself and forms the southern edge of the militarised zone. To the south (right) of the Vallum, the (later) Roman military road joining all the forts on the wall is evident in this aerial photograph. *(Photograph by kind permission of Air Images Ltd).* Inset: The massive earth-moving needed to create the 112 km length of the Vallum is evident, all the more impressive when it is realised that it was dug by hand using *dolabra*, wooden shovels tipped with iron, and moved in baskets and carts. **H)** A reconstructed turret at *Vindolanda* Roman Fort attached to a short length of the curtain wall illustrates around 30 m of the 117 km frontier that makes up Hadrian's Wall.

Ash has an air-dry density close to that of both oak and beech (650- 700 kg/m^3), and a high Modulus of Elasticity (longitudinal: 15,800 N/mm^2, radial 1510 N/mm^2) compared to other hardwoods (Desch and Dinwoodie 1996). Toughness (the resistance of wood to the propagation of cracks on sudden impact) is an essential characteristic of woods used for handles, of which Ash is a notable example. High density is a prerequisite of this strength property, as is the fibre-composite structure where relatively weak interfaces between cell-wall layers absorb energy by modifying (redirecting) the primary crack (Cook and Gordon 1964). Timber of low density, the presence of compression wood and other growth imperfections can all reduce toughness. In the case of Ash for tool handles, it is now recommended that wood having between 4 and 16 rings per 25mm should be used to avoid serious injury that can occur from failure of slow-grown stems (Desch and Dinwoodie 1996*).*

Ash is also notable for its bending properties, which depend on its structure, toughness and strength. Standard tests in which clear specimens of constant (25.4mm) thickness were subjected to bending to failure show how well Ash compared to other native species (Table 1). Comparison is also drawn with two commercially-important North American species. Tests were undertaken in which the outer 'tensile' face was supported either with or without a restraining strap, the latter being indicative of the bending undertaken in normal use.

Table 1 Limiting radii of curvature (mm) at which 5% of the population of test samples of home-grown species failed in steam bending following air-drying. (m/c not given). Data from Stevens and Turner (1970).

Species	mm (supported)	mm (unsupported)
Ash	64	300
Alder	360	480
Beech	38	330
Elm	38	340
Hornbeam	100	420
Oak (*Q. robur)*	51	330
Yew	220	420
Hickory (USA)	46	380
Sitka spruce (home-grown)	910	810

Note. American Hickory is included because it is now the preferred species for wooden tool handles throughout much of the western world.

Ash is clearly superior in unsupported bending to all these species, albeit beech and oak are perhaps more adaptable. In thin-section (3.2mm) laminates, Ash is superior to all except beech. It is easy to work, and produces few splinters. It turns well and makes strong, light furniture with a pleasing honey-coloured grain (Fig. 1 D). Its many domestic uses have been rehearsed recently (Rackham 2014: Penn 2015). Ash has long played a part in conflict (see below) and recently in two world wars (Fig. 1 E) when it formed the undercarriage skids on the

Bristol Boxkite, used as a trainer in WW1, and in WW2 formed part of the wooden structure of the De Havilland Mosquito, adding strength to wooden components in areas of high stress.

Ash management, Britain

Ash grows widely throughout Britain, performing best on deep, moist well-drained brown earths where Yield Classes 10 – 12 have been recorded (Evans 1984). Form is likely to be better in mixed than in pure stands. Hiley concentrated on growing Ash for the lucrative sports industry in south Devon (Hiley 1964*)*, and found it surprisingly hard to achieve consistently superior crops, noting that there is much to learn about the species. However, he quotes correspondence in which sales of high-quality timber were sporadic until the First World War, when the circumstances changed dramatically so that demand for the same timber from the Bristol Aeroplane Company outstripped supply.

Management of Ash by pollarding, to provide foliage as livestock feedstuff, was commonly practised over centuries (Rackham 2014). Of more significance is the management of Ash by coppicing. Rackham noted that in rural communities, Ash grows faster in coppice than as planted trees, and the resultant poles are more useful than the big tree. He suggested (Rackham 1977), from evidence collected from excavated Neolithic trackways in Somerset that coppicing started 6000 years ago. It is now clear that this form of management began much earlier (Milner *et al* 2013), since evidence similar to that found in Somerset for coppicing has been found on Mesolithic sites in Yorkshire to date from *circa* 9000BC - 7000BC, and from submerged landscapes on the edge of the English Channel (Catling 2012). By the time when farming was developing in the Neolithic in the third millennium BC, coppicing to produce a strong, resilient timber of useful and consistent size, easily harvested using simple stone tools (Coles 1979) and gathered from close-by, was therefore already well established. Its use was not confined to Britain, and has been inferred from pollen and wooden artefacts from Switzerland in the early Neolithic (Jacomet *et al* 2004). Direct evidence for coppicing other than that inferred from the shape of preserved artefacts in the prehistoric period is circumstantial at best, and no diagnostic features distinctive of coppice have been found on the ground. An added complication is that Ash is rarely common within pollen diagrams (Dumayne-Peaty and Barber 1997). However, references of the use of coppicing *per se* are not uncommon among Roman authors (McCarthy 1986). That Ash was coppiced in the Middle Ages is not in doubt (Rackham 1986), and it is readily observable today in, for example, Bradfield Woods, Suffolk, (Fig. 1 F) where some of the coppice stools are amongst the oldest living objects in Britain.

The Ash in antiquity

Ash was the chosen wood for one of the oldest anthropomorphic carved figures in Europe, found in the Somerset Levels and dated to 2100 BC (Coles 1998). It may be relevant that it is considered to be hermaphrodite (Coles and Orme 1982): apposite for a triecious species such as Ash (Thomas 2016).

In Viking mythology, Yggdrasil was a gigantic Ash that bestrode the world and heaven, with its roots in the underworld. It joined together the lands of gods and men. Its huge size and shape was strong enough to hold them apart yet keep them together in one place. It was a world of good and evil, of joy and sorrow, and it suffered more than man can understand. Yet from this vast and terrifying object, the bees feed happily on its honey dew (Brondsted 1960). Rivers that flowed from its three roots gave rise to the ancient association between Ash trees and sources of water such as wells and springs, and is one of the reasons why Ash occurs commonly in English place names (see below).

Ash had more mundane uses in Medieval England (Frazer 1949): to remove warts (in Cheshire), you were advised to rub them with bacon and slip the bacon under the bark of an Ash tree. In addition, an anti-malarial tonic was extracted from Ash bark before quinine was introduced to Europe (Lonsdale, D.L. *pers. comm.* 2016). Ash also had a special function curing rickets or rupture: a longitudinal split made in a tree was opened up, and the affected child, naked, would be passed through the fissure at sunrise at least three times. Thereafter, the split would be closed, tightly bound and sealed with clay. If the tree should die, then so would the patient, who (unsurprisingly) guarded the tree assiduously for the rest of his life. This personal attachment to Ash trees may be another reason why the name occurs with unusually high frequency among English place-names (Rackham 1986): another is the utilitarian value of the species to rural communities for the provision of small-wood and building timber, fuel, fodder and implements.

This utilitarian value of Ash is considered below in relation to the demands for wooden artefacts (particularly weapons and tool handles) in one well-researched group from the Iron Age, namely the Imperial Roman Army which occupied Britain for 350 years from AD 43. Within the first 80 years this highly-disciplined force built major infrastructures within a relatively undeveloped, rural Britain, often to standard design. They included over 2000km of permanent, all-weather roads (Margary 1967), hundreds of bridges, 300 military forts, 6 major fortresses (Webster 1969), and the 118 km (72 mile) frontier system, Hadrian's Wall (Figs. 1 G, 1 H). 20 major urban towns were constructed during that period (Wacher 1974). For a discussion on the post-conquest surveying of road alignments, see *Current Archaeology* (Issue 314) May 2016.

The Army that invaded Britain in 43 AD was composed of four legions each of 5,000 heavily-armed infantrymen with a roughly equivalent number of Auxiliary troops, the latter raised in European countries that had been absorbed into the empire. This Army crossed the English Channel in around 900 ships (Peddie 1987) and was a self-contained expeditionary force with horses, wagons and carts, tents, weapons, building tools and enough food and fodder for several days campaigning. It established a defended base near London from which it fanned out into the country probably using existing trackways (Bishop 2014), each legion and its auxiliary vexilations subduing its allotted zone. Although Britain was a land without permanent roads or bridges, in which most of the inhabitants were involved in agriculture, it was a sophisticated tribal culture with a population estimated to be between two and four million people (Cunliffe 1995), of which the majority (>90%) were rural agriculturalists (Millett 2016). Close ties with Europe gave the south-east of the country easy access to the Roman Empire, and the tribes that lived in that area were, for the most part, sympathetic to Rome and indeed some may have welcomed and aided the invasion. Further north and west, away from the continental influence, the native peoples were not compliant and a large, permanent Roman garrison was required to contain them. Thus, three legions (15,000 men), along with 35,000 Auxiliaries remained in Britain throughout the occupation (Breeze 1982): Spain, by contrast, took 100 years to subjugate but was thereafter controlled by one legion.

The tools and equipment of the legions have survived in sufficient numbers to show their diversity. Their techniques of working are well illustrated in relief carving in stone (Trajan's Column in Rome, dedicated stone slabs and altars, etc.), and are clearly evident as solid remains in many archaeological excavations in Britain. Indeed, more is probably known of the Roman Army than of other military units in Britain up to the 18th century. One of the sources of that information comes from a significant assemblage of finely-preserved military artefacts which was found in an abandoned second-century fort, *Trimontium*, at Newstead in the Scottish Borders when it was excavated 100 years ago. The photographs from the excavation report (Curle 1911) show how self-reliant the occupants of this (and of other) forts must have been, capable of manufacturing, repairing and refitting tools, weapons and equipment in wood, metal, leather and fabric in dedicated workshops (*fabricae)*, (Bishop and Coulston 2013) within the forts, and illustrate the advantages of Roman military self-sufficiency admired by contemporary writers (Vegetius, 4th Century AD).

The legions were self-sufficient partly because each man carried all the tools and equipment needed to march, fight, sleep, feed and build; clearly illustrated in Fig. 2 A, showing the members of a 'section' of eight men who operated as a unit (*contubernium)*, sharing a tent carried by a pack animal. In addition to his arms and armour, each man is recorded as carrying a saw, basket, pickaxe, a thong of leather, a hook, and three days ration: a bronze mess-tin and a kettle completed the kit, hung on a staff carried on the left shoulder (Webster 1956). The arms consisted of a pair of javelin (*pila)* and a short-sword. Attached to the staff was a wooden stake, sharpened at each end with a hand-grip in the centre, known as a palisade stake (*pilum murale)*, thought to provide a temporary defensive rampart on the march.

The auxiliaries were less encumbered than the legionaries, and were armed and protected according to the custom of the area from which they came. In general, each man carried a sword and also a spear, the latter a weapon distinct in form and function from the *pila* of the legions. The spear was a weapon for close-order fighting, and not for throwing. Ash has been the preferred haft for spears for thousands of years (Coles, Heal and Orme 1978: Travis and Travis 2014): in ancient Greece, (and indeed also in Wales) the words for spear and Ash are collocations: Homer has spears as always "ashen". Many hundreds of thousands of spear hafts would have been in use within the Roman Army of occupation, the hafts being the disposable part of the weapon. Evidence that the spear heads were valued and recycled comes from a chest of metal Roman military equipment excavated at the Roman military supply base at Corbridge, on Hadrian's Wall (Bishop and Coulston 2013). Along with armour needing repair were a number of spearheads with broken shafts, supposedly of Ash (Bishop 2016). Spear shafts could quickly be made from slender stems of coppiced Ash.

Figure 2 See next page for descriptions.

Figure 2 A) An eight-man "section" (*contubernium*) in a Roman legion prepare to move, in a drawing by Peter Connolly. Each man will carry his own equipment slung on a pole over his left shoulder. Note the two *pila* in his right hand. The equipment of the second man in line is lying on the ground. The leather 8-man tent will be carried by the donkey. Note the bundle of palisade stakes on the donkey. This picture is taken from *The Roman Army* by Peter Connolly (Macdonald 1967) *Image courtesy akg-images / Peter Connolly 2016.* **B)** Examples of *dolabra*, axes, a reaping hook and a turf cutter found during the 1910 excavation of the Roman fort at *Trimontium* in South Scotland. **C)** A Roman army axe from *Trimontium* fort, south Scotland. This axe was made by a skilled artisan. It is 250 mm long and weighs 2.7 kg. The eye is approx. 40 mm long: in a modern axe of this weight it would be at least 70 mm. *The axe is in the National Museum of Scotland, Edinburgh and the photograph is reproduced with their kind permission.* **D)** Table-top model of typical Roman Army fort, probably 2nd century and built of stone. Similar in size and layout to the earlier wooden fort at Pen Llystyn, with a perimeter of around 500 m, an area of 1.6 ha and accommodation for up to 1000 troops. **E)** A scene from Trajan's Column (Rome) celebrating the victory of Rome over the Dacians in AD 105. A legionnaire is felling trees with a *dolabra*, to clear forest and deny its sanctuary to enemy forces, for the building of a fortress, or for fuel etc. Trajan's Column, Rome. 2nd Century AD. **F)** Roman Army single-felloe cart-wheel excavated from a pit in the fort at *Trimontium*. The felloe is a single 3.1 m length of Ash, steam-bent into a circle of approx. diameter 1.0 m, encircled by an iron rim. The felloe came from a relatively slow-growing tree, and at some stage it failed at the junction with one of the spokes (inset). Both the hub and the spokes were nicely turned on a lathe, the hub of elm, and the spokes of willow. *Trimontium*, Scottish Borders, circa 150 AD. *The wheel is in the National Museum of Scotland, Edinburgh and the photographs (taken through glass) are reproduced with their kind permission*. **G)** The use of carts by the Roman Army is well attested on Trajan's Column, in Rome. In this case, carts are pictured carrying carrobalista artillery. Trajan's Column, Rome. 2nd Century AD.

In contrast to the auxiliaries' spears, the primary weapon of the legionaries was the *pilum*, of which each man carried two. This was a close-range javelin that used weight to provide its penetrative power, especially against armour (Bishop and Coulston 2013). A sharpened four-sided steel bodkin head was made at the end of a soft-metal shank, perhaps 500mm long, which in turn was riveted to the end of a weighted haft 1500mm long. At the base of the haft was a pointed metal shoe or butt, so that the weapon could be stood on end when not carried. This was a subtle weapon, particular to the Romans and well suited for use against barbarians whose natural defence to it was to raise the shield and intercept its flight. Herein lay its inherent effectiveness, since the *pilum* would readily pierce most shields, and its soft-metal shank would bend under the weight of the haft, rendering the shield useless and exposing barbarian flesh to the brutal Roman stabbing short-sword (*gladius).* As with the spear, it is probable that Ash was the preferred material for the haft, but unlike the spear the haft was a complex engineered component of value in itself. Many hundreds of thousands of these weapons would have been brought over in the invasion of 43 AD, and the weapon remained standard issue throughout the occupation. It seems likely that coppiced Ash would have been an efficient local source of supply for this weapon as well as for the spear. Key to its effectiveness was the accuracy with which it could be thrown, and the straightness of the haft was crucial. Access to a constant supply of straight stems from which the hafts could be fashioned would be a distinct advantage, and coppiced Ash the ideal choice.

Other weapons made from wood included bows and arrows, and artillery. Most formations included some bowmen, mostly from the Middle East. They used composite bows, made from horn and a variety of woods which did not seem to include Ash. Few bows survive entire. Arrows were made from many woods, of which Ash and pine are specifically mentioned (Bishop and Coulston 2013). The mobile artillery (*ballista* and *catapulta),* carried or mounted on carts, consisted of frames, mostly oak, with tensionable arms that could have been made from Ash. They shot sturdy wooden-shafted bolts, often of Ash, or stones. They are relevant because of the use of carts (see below). Each legion was equipped with 60 cart-mounted *ballista* and ten *catapulta,* each with a supporting cart carrying the ammunition (Peddie 1987*)*, making 140 carts for the artillery alone.

Among the many tools with which legionaries would have been familiar are two that relied on strong hafts: axes and pickaxes (*dolabra).* Axes (Fig. 2 C) indicate the reliance the Roman military placed on the working of timber. Their commitment and skill working in wood is exemplified in the speed with which the navy was capable of building large (>40 m long) sea-going wooden ships. The Roman author Livy noted that in 205 BC they built 30 of these ships, capable of carrying 10,000 men, from cutting timber to launching in 45 days (Pitassi 2012). On land, the use of timber was equally impressive. An "average" fort for 1000 troops, built during the pacification of Wales in the first century (Nash-Williams 1969), Pen Llystyn covered an area of 1.6 ha with a rampart length of 520 m. The external gates and watch towers along with ten barrack blocks, headquarters, workshops and hospital buildings and two granaries within the rampart were built and roofed of timber, the majority probably of oak (Hanson 1978). Assuming sizes of timber squared from logs 30 cm in diameter for the structural uprights, along with an allowance for roofing material, it is estimated that around 1000 tonnes of logs would be needed, obtained from some 2000 medium-sized trees. The harvesting, transport and preparation of these timbers was done with hand-held tools, of which the axe would have been predominant. However, the axe (Fig. 2 C) has some curious features. It is large by modern standards (250 mm long, 2.7 kg), of sophisticated construction and yet the eye (to contain the handle) is very small, presupposing a very narrow, round haft. Clearly, a very strong timber would be needed to counteract the considerable force exerted on the haft at its junction with the eye unless the haft was strengthened with a metal collar of some kind, and there is no evidence for that. The fort at Pen Llystyn is one of some 300 built in Britain (Fig. 2 D): some are smaller and a few are very much larger. A 22 ha legionary fortress for 5000 troops of about the same date, at Inchtuthil in north Scotland is estimated to have required some 16,200 cu m of timber, derived from at least 60,000 trees for its construction (Shirley 2001).

So far, I have considered the need for high-quality wooden hafts on the tools used for working timber into shapes suitable for building. It is appropriate at this point to consider also the source of the building timber itself. The sheer volumes would contradict the views of earlier archaeologists that many of these buildings were prefabricated out of stockpiled seasoned timber (Richmond 1961), and there is general agreement that structural timber was sourced locally. Additionally, great use was made in these buildings and their associated defences of wood of small diameter for the construction of woven panels to support clay or plaster walls or turf revetments. It has been estimated that in the 1.6 ha wooden fort at Carlisle, in northern England, some 50,000 poles or rods would have been needed for the six barrack blocks, let alone all the other structures and the ramparts (McCarthy 1986*)*. This material would probably have been sourced from pre-existing managed coppice woodland, created by local people to supply building materials and fuel. The large quantities of timber required for this and all the other forts that were constructed of wood along the northern frontier might indicate that the local timber resource was unlimited. In practice, this seems not to have been the case, as is shown by the inappropriate use of Alder timber in structural components of an early Roman fort (Hanson 2007). Analysis of tree pollen from around the line of Hadrian's Wall during the early part of the Roman occupation is equivocal, and shows that the vegetation was variable (Dumayne-Peaty and Barber 1998: Tipping 1997). One interpretation would suggest that the area was not clothed in dense ancient forest and wildwood: instead, it was probably quite open countryside with woodland, arable and grazing interspersed. This is relevant if it means that obtaining the timber and smallwood for the building of the large number of forts along the frontier involved long transport distances for the material, probably in carts.

Harvesting and working timber for the building of the early forts required a large and constant supply of hand tools along with their handles or hafts. There is good archaeological evidence that iron scrap was collected and reworked (see e.g. the Corbridge hoard above), and doubtless the *fabricae* would have provided new handles as well. The question as yet unanswered is where the new handles came from. Given the organisational zeal of the Roman Army, it would be surprising if local fort commanders relied on some distant depot to supply a product like a tool handle that could equally be made in the fort's workshops, from material from the Army's woodland or some other local source. There was a long tradition of coppicing within Europe, and it would not be far-fetched to suggest that the Army managed its own woodlands.

Building forts out of timber was expedient during the invasion phase, and was a tactic later employed by the Norman king William 1st following the AD 1066 invasion of England. More permanent structures would be needed thereafter, and away from the clay deposits of the south, the only alternative materials available were turf and stone. Winning and working these required equally sophisticated tools to those used for timber, and sufficient examples have survived on military sites to indicate the techniques used by the Army when creating permanent garrisons or defences. Nowhere better illustrates this work than along the Hadrianic frontier across the narrowest part of northern England, between Carlisle and Newcastle. This was a frontier with depth, made up of a number of components, some linear and others intermittent along its length. The relationships between ditch, wall, forts, Vallum and road show up well in the aerial view looking east from above Housesteads Fort (Figs. 1 G, 1 H). It was probably constructed in 10-15 years (Breeze and Dobson 1976).

In essence, the frontier controlled movement from *Pax Romana* in the south into the lands of the barbarians to the north, and *vice-versa:* it was not a defensive line like the trenches in France in WW1. Journeying from north to south a traveller would first have to negotiate a linear 'V'-shaped ditch 8 m wide, nearly 3 m deep with all its crossings opposite gates in the wall itself. The ditch ran for some 96 km, and required the digging of over 1 million cu m of soil and rock (Hill 2006). Behind the ditch, and running the full length of the frontier was the curtain wall itself. Constructed of squared rubble, the outer faces of the shaped stones were more or less roughly squared with wide joints between them. These facing stones, 25 x 30 x 25 cm, weighed about 29 kg each (Hill 2006) and 20 were needed for each square metre of wall. The wall itself varied in thickness, but averaged around 3 m and a height of 5 m. It is estimated that 24 million facing stones were required for the wall and the forts along its length Fig. 1 H). The stones were won from quarries opened as close to the wall as possible, and were worked using techniques and tools recognisable today such as bursting, splitting and dressing hammers and wedges to break the rock, and scrappling hammers, mallets, and chisels to square them. The shaped stones and the in-fill rubble probably weighed around 4 million tonnes and needed to be transported to the wall line: an operation requiring many thousands of vehicles of one kind or another (Moffat 2009). The rubble core was set in clay or in lime mortar, the latter made from limestone quarried, crushed and burnt in kilns near the wall. Scaffolding, probably of alder (*Alnus)* and Ash, gave access to the walls for building at height. It is estimated that 150,000 linear metres (1,500 cu m) of scaffold poles would have been required, and the sourcing of such large quantities of timber of adequate quality in the area of the frontier would have presented significant problems, given that fuel for the lime kilns would need to be found at the same time. The wall, and the forts, milecastles and turrets along its length, were set upon sound foundations and the areas to accommodate them levelled. The mass of earth to be dug and shifted has not been estimated. But the primary tool for the job was the *dolabra*, the standard, multi-purpose pick-axe/mattock digging tool carried by each soldier as routine (Fig. 2 E). Many hundreds of thousands of these beautifully designed, ergonomic tools would have been needed. Fine examples exist (Fig. 2 B), and they show the same puzzling characteristic as the axe head, having a very small eye for the haft compared to the modern pick-axe handle. These tools were used for levelling, for felling trees and probably also for cutting turfs. *In extremis* they were very effective close-quarter weapons.

Set some distance behind the wall, and creating a further impediment to accessing the wall itself and its buildings, a complex linear feature was constructed. Known as the Vallum (Fig.1 G inset), it demarcated the southern boundary of the military zone that was the frontier. It consisted of a ditch some 3 m deep and 6 m wide, the upcast spread as 1.5 m high mounds along both sides to form an obstacle over 40 m wide to all traffic, only crossed at 10km intervals. Over its entire length of 112 km, the volume of earth and rock excavated and spread was around 1.5 million cubic metres.

At a later date, running roughly parallel to the Vallum and south of it, a road was constructed to link all the parts of the frontier together, and it serves to illustrate in general the efforts made by the Romans to create a network of all-weather routes linking the occupied areas of Britain. Roads varied in width, but most contained the same elements, namely a well-tamped foundation of coarse rubble, boulders or, in wet areas, log corduroy set in a shallow ditch the width of the metalled surface. This ran down the centre of an area cleared of obstructions such as trees, rocks and stumps, along which the roadside drains were cut. Depending on the nature of the subsoil, material from the drains was used to form the running surface or *agger* which was well compacted and cambered to provide water run-off into the drains.

Using 18th century data on the building of military roads in Scotland, Bishop (2014*)* estimated a rate of construction of about 2 man-years per km of road. As with the construction of the ditch and Vallum on Hadrian's Wall, this work depended on the use of hand-held tools, and on waggons or carts to transport the building materials as required. By the end of the Roman occupation, it is estimated that some 12,000 km of roads had been built in Britain.

In each of the cases described above, where large masses of the building materials timber, wood, rock, limestone, sand and gravel had to be moved, it seems likely that wooden carts or waggons would have been employed. Three types of cart and their maximum loads were defined in the 4th century (Hill 2006*)*: *Angaria (*490 kg*)*, *Raeda* (328 kg) and *Carus* (164 kg), but there are no data on the waggons used by the Roman military in Britain. The use of carts for the transport of a wide variety of goods and equipment is well documented on Trajan's Column (Fig. 2 G), and a pair of wheels remarkably well preserved (Fig. 2 F) was excavated at *Trimontium* (Curle 1911). These were iron-shod, roughly 1 m in diameter, with 10 spokes of willow fitted to the turned elm hub with square mortises. The spokes were fitted by means of round mortises into the felloe. Usually made from a number of curved lengths of wood, the felloe of this wheel is of a single 3 m length of Ash, steam-bent into a circle with the ends joined by an iron plate. This is a well-attested technique of wheel making, reaching back into the Iron Age (Crouwel 2012) in Europe and, using Ash, to the 5th Century BC in Scotland (Carter, Hunter and Smith 2010). While the techniques associated with the making of spoked wheels with several lengths of felloe "*represents one of the masterpieces of prehistoric woodworking, combining extreme lightness with strength and* *durability, blending the different properties of different woods …to achieve the best possible combination…"* (Coles, Heal and Orme 1978*)*, bending a single 3 m length of Ash into a perfect circle and then fixing it accurately to the hub by means of mortised spokes requires a technique that few today can aspire to. In the *Trimontium* wheels, the Ash was sufficiently well preserved to permit some observations to be made on it. The felloe was split from a clear length of Ash, with about 19 annual rings set within its 10 cm width (i.e. an annual growth rate of around 5 mm). Because the wheel is encased in a glass cabinet, it was not possible to make observations on the profile of the felloe, but from the photograph it looks roughly rectangular. The distortion of the annual rings around some branch initials would suggest that the bend was radial, with the sapwood on the outer edge, next to the iron tyre. The felloe shows clear evidence of failure, where one of the spokes has sprung out of its mortise (Fig. 2 F inset). This distortion shows signs both of compressive and tensile failure. Such failure might occur if the wheel was over-loaded and hit an obstacle. Once broken, the wheel along with another was discarded.

This technique of making single-felloe wheels lasted into the Middle Ages in Britain, when the tradition was lost (Jenkins 1966). However, it had lasted for nearly 1000 years, and presupposes that the mysteries of the technique were handed down from father to son and that an adequate supply of high-quality fast-grown Ash suitable for this severe bending was readily available locally. Given that such material would be available from coppiced woodlands, but only if the coppice was regularly cut and managed (Anon 1956) raises the question of its disappearance from the countryside. As an example of the decline in coppicing as a means of managing woodland, it is instructive to see that in 1952, of a total of 47,500 ha of hazel coppice in England, 42,500 ha (89%) was classed as unworked. A system that had been successfully used for hundreds if not thousands of years up until a century ago has virtually disappeared, along with most of the skills and knowledge that underpinned it.

We have no evidence that the Roman Army created and managed any woodland in Britain to provide it with the timber resource that, in its expansion phase, it so clearly needed. But the Army's attention to detail, its ability to plan ahead and its pragmatic organisation would make it seem unlikely that it did nothing to safeguard an essential commodity as important as timber. That a system of coppice woodland management developed in England after the Norman invasion that has, in tiny parts (eg Bradfield Woods, Suffolk Fig. 3 C) survived to today probably has nothing to do with the Roman Army.

For most of the 1600 years that have passed since the Roman occupation of Britain came to an end, the population of these islands has been dependent on Ash to provide it with the tools needed to work the land. By the time of the Doomsday census of 1086, around 90% of the population (of 2 million) did not live in towns but were involved in agriculture. By 1850, only half the population depended on agriculture but, since it had risen to 27 million, this left perhaps five million and their dependants working in farms and forests with hand-held tools (Fig. 3 B) whose handles and hafts, if made from Ash, were strong, flexible, resilient, free from splinters and locally available. Ash met those requirements better than any other species commonly available in Britain.

Ash today

The industrialisation of farming and the reliance on coal, oil and electricity instead of wood for fuel has reduced the demand for Ash in the last century or so. The species is now valued more for its conservation attributes than for the properties of its timber. However, there remains a small market for sports and domestic goods which has been recently described by Rackham (2014) and Penn (2015). Notably, good quality Ash is used today in bespoke Morgan Cars, hand-made in Malvern, using Ash for the frame on which the aluminium bodies are fitted. This work (Fig. 3 A) requires standard components accurately machined and bent to accommodate the tight tolerances needed in a light sports vehicle capable of 155 mph (255 kph). It is a direct descendent of earlier carts (Fig. 3 A). The finest of bent-wood furniture uses Ash: Dan Hussey's complex furniture (Fig. 1 D) is constructed from planks split from coppiced Ash from Bradfield Woods (see above). Both these industries continue the tradition of matching the product to the appropriate material.

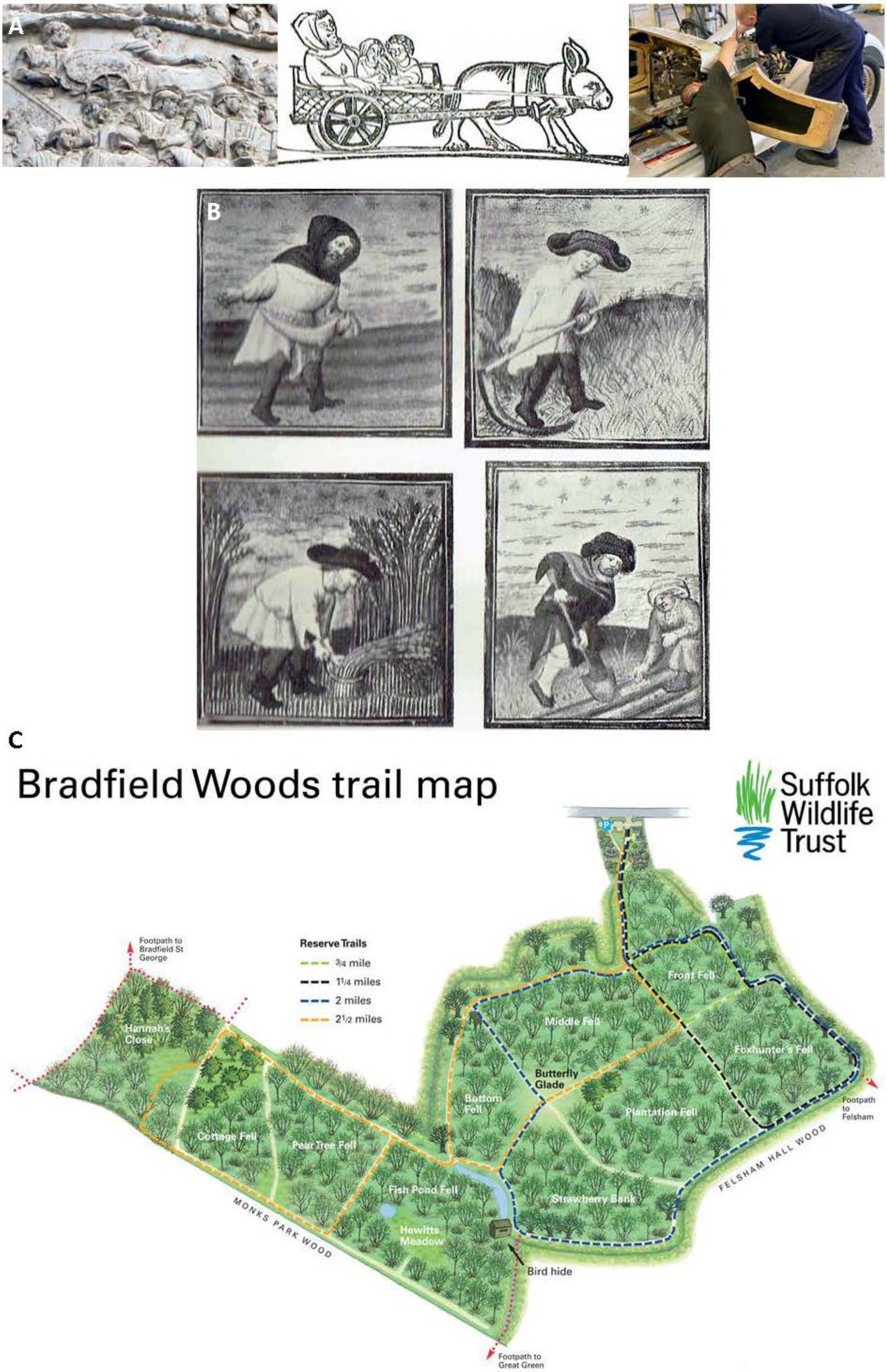


Figure 3 See next page for descriptions.

Figure 3 A) Each of these images is separated by about 1000 years, yet each involved Ash in some form of transport. First, the Emperor Trajan's troops use a mule-drawn cart to move what looks like a tent in the campaign against the Dacians, 2nd Century AD. From Medieval England a family is transported in a dog cart. From modern England, a Morgan sports car is created using Ash for the framework at the Malvern factory, 2013. **B)** Medieval husbandry relied on hand-held implements for almost all the work needed to keep body and soul alive. **C)** Bradfield Woods, in Suffolk, England has been a source of supply of coppiced Ash for at least 1000 years, the long, clear stems being used locally to make hay rakes, scythe handles and fine bent furniture. The woods are divided into at least ten sections, to ensure a range of ages for continuous coppicing. *Map courtesy Suffolk Wildlife Trust.*

The utility of Ash is summed up in a recent poem (Rawling 2009) which celebrates a crucial agricultural practice, commonplace up to the 1950's, of stacking harvested wheat sheaves (*bundles)* in a barn for later threshing:

Bright prongs pierced and unpicked, ash handles
bent, they launched the bundles we embraced,
laid in a level course, crammed into corners,
trod tight. Crispness, countless swathes
of kizzened grass pricked our skin,
there was wildflower scent in our nostrils.
When our heads bumped the slates
We came down the ladder in triumph.

What relevance does past use have on the survival of the species now?

In any project to preserve the gene pool of our native Ash against the possible extinction of the species from the combined onslaught of Ash Dieback (*Hymenoscyphus fraxineus* Queloz *et al.)* and of the Emerald Ash Borer (*Agrilus planipennis* Fairmarie), it is perhaps necessary to consider to what extent the past use of Ash may have affected the genetics of the present population.

There seems to be wide consensus that Ash has been managed by coppicing in the past, to provide easily-harvested wood of appropriate size and strength to meet the local demands for tool handles, weapons, building materials and fuel. The extent to which coppicing involves selection of desirable traits is perhaps not clear. But given the relationship between growth rates, strength and flexibility described above, it would be surprising if the Ash population in Britain has not been subject to structural "improvement" over the past few thousands of years, making it better suited to the needs of a pre-industrial agrarian society which would have valued rapid growth and relatively short rotations. In addition, it can be argued that the immense age of some of the coppice stools will have tended to influence genetic variation among the existing population. And because Ash is dioecious, it is also possible that selection may have been biased towards either predominantly male or female trees if there are differences in growth patterns between the sexes. Whatever the truth, it is worth bearing in mind that for several thousands of years, Ash had a special place in our history and it will be a tragedy if it has come to an end.

Acknowledgements

Keith Barber, Mike Bishop, Peter Fordham, Peter Gasson, Bill Hanson, Fraser Hunter, Robert Hurford, Dan Hussey, Chris Stringer, Dirk Sporleder and Alison Sheridon have patiently submitted to my interrogation and given me copies of their papers. Angela Pitt and David Lonsdale kindly reviewed the text. Margaret Wilson, National Museums Scotland, generously gave permission for the use of my photography of the Museum's exhibits, and the Suffolk Wildlife Trust made their map of Bradfield woods available. David Lonsdale's expert opinion on the nature of the failure of the wooden wheel was particularly appreciated.

Except where credited, all the photographs are the author's, as are all the errors.

References

Anon. 1956. *Utilisation of Hazel Coppice*. Bulletin No 27, Forestry Commission, HMSO, Lond. 34pp.
Bishop, M.C. 2014. *The secret history of the Roman roads of Britain.* Pen and Sword Military, Barnsley. 210 pp.

Bishop, M.C. 2016. Pers. Comm. Feb 2016.

Bishop, M.C. and Coulston, J.C.N. 2013. *Roman Military Equipment,* 2nd Edition. Oxbow, Oxford 322pp.

Brazier, J.D. 1990. *The Timbers of Farm Woodland Trees*. Bulletin, Forestry Commission No 91, HMSO, Lond. 8pp.

Breeze, D.J. 1982. *The Northern frontier of Roman Britain*. Batsford, Lond. 188pp.

Breeze, D.J. and Dobson, B. 1976. *Hadrian's Wall.* Allen Lane. 324pp.

Brondsted, J. 1960. *The Vikings*. (trans. E. Bannister-Good.) Penguin Books, Lond. 320pp.

Carter, S., Hunter, F. and Smith, A. 2010. A 5th Century BC Iron Age Chariot Burial from Newbridge, Edinburgh. *Proc. Prehistoric Society* **76:** 31-74.

Catling, C. 2012. Bouldnor Cliff: a glimpse into the Mesolithic. *Current Archaeology* **262***:* 31-37.

Coles, B. 1998. Wood species for wooden Figures: a glimpse of a pattern. In: Gibson and Simpson (eds.) *Prehistoric Ritual and Religion: Essays in honour of Aubry Burl.* Sutton Publishing.

Coles, J. 1979. An experiment with stone axes. Pages 106-107 in: McK Clough and Cummins (eds.) *CBA Research Report No 23: Stone Axe Studies.* Council for British Archaeology, Lond. 137pp.

Coles, J.M. and Orme, B.J. 1982. *Prehistory of the Somerset Levels.* Somerset Levels Project 1980. 64pp.

Coles, J.M., Heal, S.V.E. and Orme, B.J. 1978. The use and character of wood in prehistoric Britain and Ireland. *Proc. Prehistoric Society* **44,** 1-45.

Cook, J. and Gordon, J.E. 1964. A mechanism for the control of crack propagation in all brittle systems. *Proc. Royal Soc.* A **282**: 508.

Cooney, G. and Mandal, S. 1998. *The Irish Stone Axe Project, Monograph 1.* Wordwell Ltd, Bray, Ireland. 220pp.

Crouwel, J.H. 2012. *Chariots and other wheeled vehicles in Italy before the Roman Empire.* Oxbow, Oxford 234pp.

Cunliffe, B. 1995. *Iron Age Britain*. Batsford, Lond. 128pp.

Curle, J. 1911. *A Roman Frontier Post and its People.* Maclehose, Glasgow. Vols I and II.

Desch, H.E. and Dinwoodie, J.M. 1996. *Timber: structure, properties, conversion and use.* Palgrave Macmillan, 306pp.

Dinnis, R. and Stringer, C. 2014. *Britain: one million years of the human story*. Natural History Museum, London. 152pp.

Dumayne-Peaty, L. and Barber, K. 1997. Archaeological and environmental evidence for Roman impact on vegetation near Carlisle, Cumbria: a comment on McCarthy. *The Holocene* **7** (2), 243-246.

Dumayne-Peaty, L. and Barber, K. 1998. Late Holocene vegetational history, human impact and pollen representivity variations in northern Cumbria, England. *Journal of Quarternary Science* **13** (2), 147-164.

Evans, J. 1984. *Silviculture of Broadleaved Woodland.* Forestry Commission Bulletin 62. H.M.S.O. Lond. 232pp.

Frazer, J.G. 1949. *The Golden Bough*. Macmillan, Lond. 756pp.

Green, H.S. 1978. Late Bronze Age wooden hafts from Llyn Fawr and Penwyllt and a review of the evidence for the selection of wood for tool and weapon handles in Neolithic and Bronze Age Britain and Ireland. *Bull. Board of Celtic Studies* **28**: 136-141.

Hanson, W.S. 1978. The organisation of Roman military timber supply. *Britannia* **9**: 293-305.

Hanson, W.S. 2007. *Elginhaugh: a Flavian fort and its annex*. Britannia Monograph Series No. 23, S.P.R.S., London. 686pp.

Harding, A. and Young, R. 1979. Reconstruction of the hafting methods and function of stone implements. Pages 102-105 in McK Clough and Cummins (eds.) *CBA Research Report No 23: Stone Axe Studies.* Council for British Archaeology, Lond. 137pp.

Hiley, W.E. 1964 . *A Forestry Venture.* Faber and Faber, Lond. 240pp.

Hill, P. 2006. *The construction of Hadrian's Wall.* Tempus, 160pp.

Jacomet,S., Leuzinger,U.und Schibler,J. 2004. *Die jungsteinzeitliche Seeufersiedlung Arbon, Bleiche 3. Umwelt und Wirtschaft.* Sonderdruck-Synthese aus Archäologie im Thurgau 12. Frauenfeld.

Jenkins, J.G. 1966. *Traditional Country Craftsmen*. Routledge & Kegan Paul, Lond. 236pp.

Maigrot, Y. 2011. Neolithic polished stone axes and hafting systems: technical use and social function at the Neolithic lakeside settlements of Chalain and Clairvaux. Pages 281-294 in: Davis and Edmonds (eds.) *Stone Axe Studies III*. Oxbow Books, Oxford. 441pp.

Margary, I. 1967. *Roman Roads in Britain*. John Baker, Lond. 546pp.

McCarthy, M.R. 1986. Woodland and Roman Forts. *Britannia* **17,** 339-343.

Millett, M. 2016. How Roman occupation redrew an iron-age landscape. *Current Archaeology* **314**, 26-31.

Milner, N., Taylor, B., Conneller, C. and Schadia-Hall, T. 2013. *Star Carr. Life in Britain after the Ice Age.* Council for British Archaeology, York. 112pp.

Moffat, A. 2009. *The Wall: Rome's greatest frontier.* Berlinn, Edinburgh 270pp.

Mytting, L. 2015. *Norwegian Wood. Chopping, Stacking and Drying Wood the Norwegian Way*. MacLehouse Press, London. 192pp.

Nash-Williams, V.E. 1969. *The Roman frontier in Wales*. University of Wales Press, Cardiff. 206pp.

Peddie, J. 1987. *Conquest: the Roman invasion of Britain.* Sutton Publishing, Glos. 214pp.

Penn, R. 2015. *The Man who Made Things out of Trees*. Particular Books, 234pp.

Pitassi, M. 2012. *The Roman Navy.* Seaforth, 184pp.

Rackham, O. 1977. Neolithic woodland management in the Somerset levels: Gavin's, Walton Heath and Rowland's tracks. In Coles *et al* (eds.) *Somerset Levels Project* No. 3, 65 – 72.

Rackham, O. 1986. *The history of the countryside.* Dent, London. 445pp.

Rackham, O. 2014. *The Ash Tree.* Little Toller Books, Dorset. 178pp.

Rawling, T. 2009. *The Barn.* In Slater and Rawling (eds.) *How Hall: Poems and memories, a passion for Ennerdale.* Lamplugh and District Heritage Society, 90pp.

Richmond, I.A. 1961. Roman Timber Building. In Jope (ed.) *Studies in Building History.* Odhams, Lond. 287pp.

Sheridan, A. 1992. Scottish stone axeheads; some new work and recent discoveries. In Sharples and Sheridan (eds.) *Vessels for the Ancestors: Neolithic of Britain and Ireland*. EUP, Edinburgh. 416pp.

Shirley, E. 2001. *Building a Roman legionary fortress.* Tempus, 160pp.

Stevens, W.C. and Turner, N. 1970. *Woodbending Handbook.* H.M.S.O. 109pp.

Thieme, H. 1997. Lower Paleolithic hunting spears from Germany. *Nature* **385**: 807-810.

Thomas, P.A. 2016. Biological Flora of the British Isles: *Fraxinus excelsior.* *Journal of Ecology* in press. doi 10.1111/1365-2745.12566.

Tipping, R. 1997. Medieval woodland history from the Scottish Southern Uplands. In Smout (ed.) *Scottish Woodland History.* Edinburgh, 215pp.

Travis, H. and Travis, J. 2014. *Roman Shields.* Amberley Publishing, Stroud. 192pp.

Vegetius, P. Fl. *Epitoma rei militaris.* **II**, 11.

Wacher, J. 1974. *The towns of Roman Britain.* Batsford, 460pp.

Webster, G. 1956. *The Roman Army: An Illustrated Study*. Grosvenor Museum, Chester, 52pp.

Webster, G. 1969. *The Roman Imperial Army of the first and second centuries AD.* Adam and Charles Black, Lond. 330pp.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 15 – 26.

Who has a stake in ash dieback? A conceptual framework for the identification and categorisation of tree health stakeholders

N. DANDY [1]*, M. MARZANO [2], E. F. PORTH [3], J. URQUHART [4], C. POTTER [4]

[1] The Plunkett Foundation, The Quadrangle, Woodstock, UK, OX20 1LH, [2] Forest Research, Northern Research Station, Roslin, UK, EH25 9SY, [3] University of Surrey, Guildford, UK, GU2 7XH, [4] Imperial College London, South Kensington Campus, London, UK, SW7 1NA
** Corresponding author: norman.dandy@plunkett.co.uk*

Abstract

Stakeholder engagement is increasingly recognised as an essential component of environmental management. But what does it mean to have a 'stake' in tree health? In this chapter we use case-study analysis to explore the stakeholder concept in relation to tree health. We develop a framework to underpin better understanding of the stakeholder landscape in tree health and through which to categorise individuals and groups within it. This chapter highlights how the framework can facilitate more effective engagement and communication that is sensitive to the particular needs of different stakeholder groups, with a specific focus on the case of ash dieback (*Hymenoscyphus fraxineus*) in Great Britain. We use it both to improve understanding of how the outbreak developed over time, and to identify the roles of a diverse range of stakeholders as they became involved at different points in the outbreak. Critical reflection enables lessons to be learned for future stakeholder engagement, such as recognition of how stakeholder engagement changes over the course of an outbreak, identification of potential key stakeholder groups that may be overlooked or difficult to access, and which stakeholders are likely to be most influential in driving or facilitating behaviour change.

Keywords: Tree health; biosecurity; stakeholder analysis; forest governance; *Hymenoscyphus fraxineus*; ash dieback.

1. Introduction

Stakeholder analysis has become firmly established as a core component of environmental and natural resource management (Reed et al 2009). Various methods have been applied in sectors such as forestry (Sandstrom et al 2016), fisheries (e.g. Maguire et al 2012), climate change (e.g. Van de Kerkhof 2004), and water management (e.g. Lienert et al 2013), with the aim of improving managers' knowledge and understanding of who has a 'stake', how, and why. Some biosecurity research has sought to improve understanding of stakeholders and made use of stakeholder analysis tools, primarily in relation to animal health (e.g. Gilmour et al 2011; Hernández-Jovera et al 2012), but also occasionally plant health (Mills et al 2011; Marzano et al 2015).

Originating with Freeman (1984), we define a stakeholder as *any individual or organised group (i.e. business; organisation; association) who can affect or be affected by a decision, action or policy*. The two dimensions of stake are conceptualised as relationships of *influence* (can affect) and *interest* (can be affected by). In recent years, seeking engagement with stakeholders has become a prominent feature of tree health policy in the UK and beyond (e.g. Defra and FC 2011; Defra 2014). However, to date the stakeholder label has been applied in a relatively narrow sense, to organisations (and some individuals) with a relatively strong and direct link to the issues (e.g. forestry and horticultural industries; landowning actors). Tree health 'stakeholders' are is often explicitly juxtaposed with 'the public', to denote this more direct relationship (e.g. Defra and FC 2011). Although these actors are clearly important within tree health, even the most cursory examination of the sector will reveal many others who are also bound up in this arena and that many actions taken by those beyond this narrowly defined group can have a critical impact on the outcomes of tree health policy and management. Recent experience of outbreak events ash dieback (Heuch 2014), oak processionary moth (*Thaumetopoea processionea*) (Tomlinson et al 2015) and Asian longhorn beetle (*Anoplophora glabripennis*) (Porth et al 2015), has demonstrated the limits of these traditional models of tree health governance and management, and identified the need for approaches which integrate efforts across governmental, third sector and private stakeholders. Current UK policy emphasises a primarily instrumental (Reed et al 2009) approach to non-governmental stakeholders focused on enabling them to play an increased role in tree health and biosecurity. This consists of, for example, providing more and improved opportunities for pest surveillance and monitoring (including citizen science approaches), along with clearer guidance on ensuring biosecure practices in such as plant procurement.

This chapter critically reflects on 'stakeholder landscape' of ash dieback in the UK. This comprises a wide-ranging set of stakeholders, as well as their associated interests and influences. Through a case-study analysis, we provide a novel conceptual framework to address the question 'Who has a stake in tree health?'. Environmental decision-makers can use this framework to improve stakeholder engagement by identifying and categorising the many actors connected to tree health: clarifying their current and potential roles, exploring how stakes can change through the phases of an outbreak, and better understanding the range and type of social impacts that tree pests and diseases can have.

The framework presented in this chapter was developed through case-study analysis of five forest pests or diseases drawing on a number of data gathering exercises that included interviews with key individuals; documentary reviews including scientific papers, policy documents, and grey literature; and a detailed analysis of the supply chains and pathways involved in the spread of tree pests and diseases that focused on both the case study pests and diseases *and* on the primary means of spread associated with them – namely, wood packaging used within international trade, woodfuel, and the live plant trade.

2. Tree Health Stakeholdership: A Conceptual Framework

2.1 Categorising Tree Health Stakeholders

In tree health a stakeholder may be defined as *an individual or organisation that can either affect or be affected by a forest pest or disease outbreak*. A stakeholder's 'influence' can be defined as *its capacity to affect an outbreak's occurrence, trajectory or outcomes*. A stakeholder's 'interest' can be defined as *its susceptibility to losses or capacity for gains resulting from an outbreak's occurrence or outcomes, or associated activities* (e.g. trade). The categories described in this framework are not mutually exclusive and single stakeholders can have *both* influence on and an interest in an outbreak, as well as various forms of influence and/or interest..

2.1.1 Categories of Influence – Capabilities, behaviours and practices

We have identified five categories of capability, behaviour and practice through which individuals and organisations can affect the occurrence, trajectory and outcomes of forest pest outbreaks. These are *vectors*, *governors*, *managers*, *monitors* and *networkers*.

Vectors are perhaps one of the most obvious groups of tree health stakeholders as they consist of people whose activities, either intentionally or otherwise, physically spread a pest or disease from one area to another. They include businesses or individuals who satisfy customer requests by moving commodities from infested areas to un-infested areas through key supply chains such as woodfuel, timber, live plants or trade using wood packaging. It also includes those who can transfer a pest directly between trees or forests on contaminated equipment or footwear (e.g. arboriculture workers, foresters, tourists or outdoor recreationists). This category of stake can also include many individuals, businesses or organisations involved in supply chains that have no substantive connection to trees or other plants, including transport companies and others.

Governors are those with the capacity to set formal and informal rules and regulations which impinge on the behaviour and practices of others in the tree-health arena. Such rules can originate from trade agreements, legislation, institutional/organisational arrangements, or industry best practice initiatives and codes. They define, to varying degrees of exactness, the actions of intrinsic relevance to tree health such as inspection, phytosanitary practices, purchasing, forest management, and the allocation of resources. It is not only the form of these behaviours and practices that can be set, but their presence or absence (e.g. where trade rules prevent inspection of commodities at certain points in their movement, or direct inspections towards certain commodities and not others).

Managers are individuals or groups that possess the specific technical skills and capabilities required to deal with a tree pest or disease outbreak. Those with resources to manage pests at border points (e.g. treatment of infestation or infection) also fall into this category. The stakeholdership in this category is particularly sensitive to the pest or disease and the form of management or control required. For example, where a management response requires public information provision, this may bring actors such as local authorities into the stakeholder landscape. However, if a programme of sanitation felling is deemed necessary, forestry and / or arboricultural contractors would be key 'managers'. Forest planners and business managers responsible for adapting timber harvesting strategies in response to outbreaks are a further group of stakeholders in this category.

Monitors are those with the knowledge required to predict, detect, identify, or otherwise understand pests and pest outbreaks. This includes many actors in the science sector such as plant pathologists, entomologists, epidemiologists and modellers working in universities and government institutions, but can extend beyond this to include 'lay' expertise held by non-governmental organisations and tree professionals, as well as those mobilised by 'citizen science' initiatives. These stakeholders design and undertake surveillance work, establish routines and tests to identify pests, and conduct research. They also often work closely with 'managers' in implementing responses to outbreaks through, for example, offering advice about the biology, movement and ecology of the pest.

Networkers are key to communication amongst stakeholders. They are actors with the capacity to disseminate information and key messages to and from other actors relevant to tree health. This stake is created in part by the abilities of particular individuals or groups, but also, critically, by virtue of their position, role and level of trust within established social networks. 'Networkers' are, therefore, similar to some 'governors', in that they do not necessarily have a strong direct link to trees or pests, but they can play an important role in raising awareness and exchanging knowledge . Trade bodies and associations are an example of this.

2.1.2 Categories of Interest

We have identified five categories of susceptibility to loss, or capacity for gain, resulting from a tree pest or disease outbreak, its management, outcomes, or its associated / contributory activities. Within the framework there are three core categories that describe how stakeholders can have a 'losing' stake: they can be *'value', 'cost'* and *'collateral' losers*. Conversely, pest and disease outbreaks may present significant opportunities for gain for some stakeholders in what we term '*outcome winners'* and '*contributors'*.

Value losers are stakeholders for whom the value of relevant trees and forests is reduced by pest or disease outbreaks. Trees can provide considerable economic, social and environmental values to those who own, use, view or otherwise engage with them and the spaces around them. Pests and diseases may negatively affect, for example, the value of timber, the aesthetic appearance of forest landscapes, or the biodiversity value of woodlands. This category can, therefore, conceivably consist of a very wide range of individuals and organisations. Outbreaks may in some cases not only act to reduce value, but also release the value of forests at unexpected times. This is particularly the case in relation to economic value through, for example, the need to conduct harvesting operations earlier than planned.

Cost losers are those who bear the economic costs of responding to tree pests, which are incurred in addition to the loss of existing value (e.g. paying for labour and materials required for felling, financial compensation for damage caused by outbreak management, and cost of information dissemination). This category is most likely to include landowners and public bodies who are legally responsible for the management of pests or diseases affecting their property. Forest and tree owners will often be both value *and* cost losers.

Collateral losers are individuals or groups who lose indirect, non-tree related value, as a result of outbreaks and their management. This category represents a type of social cost that can include reputational loss amongst the contractors, consultant specialists, public bodies and others resulting from poor outbreak management. The impacts can cascade well beyond the immediate location of the particular outbreak and affect the incomes and livelihoods of many individuals and groups. For example, they might reduce the attractiveness of forest locations for recreation, consequently affecting livelihoods dependent on local tourism.

Outcome winners are those for whom outbreaks have benefits or are perceived positively. For example, arboricultural and forestry businesses can obtain income for control work (surveys, sanitation felling, pesticide application), along with the manufacturers of management materials and equipment. Some businesses may be created (or are able to adapt) to exploit the specific economic niches or opportunities created by outbreaks. This would include those offering legal or technical advice, or diagnostic services, along with those marketing particular types of wood products generated by outbreaks. Further to this, scientists obtain research contracts to investigate pests and diseases, and agri-chemical businesses generate revenue from the development and use of pesticides.

Contributors are individuals and groups engaged in activities that are beneficial to them, but which are implicated in the occurrence of pest outbreaks. 'Contributory' activities include trade and consumption, and occur consistently. Perhaps the clearest examples of stakeholders in this category are those engaged in trade of particularly high-risk commodities transported in wood packaging materials and/or live plants (producers; suppliers; transporters; traders; sellers; customers). Some, but not all, of these actors may also be implicated as ‘vectors’. Also, given that production-oriented forestry has created and benefitted from specific types of forest (often even-aged, non-native monocultures) that may be a host for pests and/or more vulnerable to pest outbreaks, it may also be possible to conceptualise some forest managers as falling into this category.

2.2 How stakes change

The tree health stakeholder landscape is dynamic and continually changing. The capacity of individuals and groups to affect pests and diseases, as well as their vulnerabilities to them, alter with time as stakeholder influences and interests shift, and as the outbreak progresses. The main driver of this dynamism is the development of outbreaks over time, from mobilisation of the pest or disease from its native range, through its introduction to a new environment, to societal adaptation to its presence. Stakeholdership also changes with levels of awareness and activism: individual and group perceptions and understandings shift as they are brought into, or choose to become more active in, the outbreak.

Outbreak stage

Our framework features a novel eight-stage model to reflect how outbreaks evolve over time (Table 1). This builds on the foundation provided by biological models of invasion (e.g. Heger and Trepl 2003; Williamson 2006), but expands their scope to encompass human actions relevant to tree health. Within biological models the number of pests on a pathway reduces at each stage prior to introduction, subsequently increasing again once in their new environment. To some extent the breadth and depth of the stakeholder landscape mirrors this, with the least number of stakeholders and most concentrated suite of relevant human behaviours occurring at the point of introduction. The model presented as part of our conceptual framework not only considers the human dimensions of these biologically oriented phases of pest invasion, but adds further human oriented stages. It is critical to recognise that numerous human activities are relevant to pest invasions both before and after the initial mobilisation of the pest or disease.

Table 1 Stages of Tree Health Stakeholdership

Stage Name		Description
Pre-pathway		Pest exists in its native range. Activities are taking place that form a potential pathway.
Introductory Pathway	Mobilisation	Pest is mobilised from its native location and is attached to a mobile medium.
	Introduction	Pest is moved and introduced to a new location where it was previously not present and potential host species exist.
	Release	Pest escapes containment or is released from attachment to mobile medium.
Establishment		Pest establishes a self-sustaining population in the new environment.
Spread		Pest spreads or disperses to new localities within the new environment.
Containment & Mitigation		Actions taken to halt or contain pest spread and/or mitigate its impacts.
Adaptation		Stakeholders adapt to (learn to live with) a pest’s presence and impacts.

A number of stakeholders are engaged in *pre-pathway* activities that affect the context for outbreaks by establishing or mitigating pathways and/or potential host forests. This stage does not feature in biological models of invasion. Forestry and other commercial enterprises (e.g. quarrying of stone for export utilising wood packaging) form the beginning of supply-chains, whilst everyday economic practices such as purchasing and consumption provide an end-point. A very broad range of activities undertaken in the pre-pathway stage set the context in which a pest or disease will be managed. Research, policy formulation, the establishment of biosecurity practices, and the marketing of commodities are just a few of the critical activities undertaken prior to specific outbreak events. Also important at this early stage are relatively generic and disparate processes such as problem definition, the formation of attitudes towards outbreaks, and the structuring of related political debate. Given the above, stakeholdership at this stage is very broad and can be both general (i.e. related to tree health broadly) and specific (i.e. associated with individual pests or diseases). Identification of the stakeholder landscapes of specific pests is possible either subsequent to an outbreak (by reconstructing the chain of events leading to a pest introduction) or in advance (through dedicated stakeholder analysis associated with a pest risk analysis). The identification of stakeholders in this stage is critical to the success of preventative measures.

The initial step in a pest's movement is its physical attachment to a carrier within its native range and *mobilisation* onto an *introduction pathway*. Again, this stage is rarely included in biological models that typically start at the point of introduction, however Colautti and MacIsaac (2004: 137) note that an organism is 'taken into the transport vector … usually by humans'. This human involvement in mobilisation may be relatively direct and intentional (e.g. via plant collection and movement) or indirect and unintentional (e.g. carrying the pest on vehicles, clothing or packaging). Alternatively, mobilisation may have no human involvement and be due to 'natural' spread (e.g. by wind dispersal). Phytosanitary actions (e.g. heat treatment of wood packaging through ISPN15 regulation) are particularly relevant during this stage. The next stage sees *introduction* (movement) to a new location where potential host species exist and can be reached by the pest or disease. Human involvement at this stage is potentially substantial, with various international trades and other movements (e.g. tourism) playing a central role. Border security is critical at this stage, with stakeholders implementing numerous security and surveillance practices designed to prevent pest introduction. Movement alone, however, is not sufficient to create an outbreak. At least one organism needs to 'escape' or otherwise be 'released' from its transporting medium and reach a viable host species. Human actions, albeit of a very much reduced set of stakeholders, are once again likely to be of significance during this *release* stage, including, for example, logistics companies handling the transporting medium. The release of a pest or disease can occur on borders and at ports of entry, although with contemporary modes of containerised transport, release or escape may potentially occur anywhere a container is opened and infested or infected commodities are exposed to the environment.

Following the establishment of a self-sustaining population, a pest species may spread in order to colonise further new localities. The boundary between the release and spread stages are not, however, always clear. The dispersal of a pest from a single introductory point may involve either further movement within a territory on the pest's original medium and pathway, or movement ('natural' or human-mediated) from an established population, or both. In the case of the former (onward movement on the original medium), this could conceivably result in two widely separated 'outbreaks' – albeit affecting a similar and overlapping set of stakeholders. Although ecological, climatic, topographical or other environmental factors may well limit pest spread, human factors can once again be causal during this stage. These may include plant buying and selling, forest recreation activities, and inspection and surveillance. As individuals and organisations (such as woodland owners, gardeners, and countryside visitors) begin to experience the consequences (impacts) of a new outbreak during this stage, new stakes are activated.

The successful spread of a pest species from an established population is generally the final stage in biological models of invasion. However, moving beyond the analysis of purely biological factors leads to the identification of outbreak stages wherein biological factors may not alter substantively, but human dimensions - and therefore stakeholder constellations - may change significantly. The first such stage can be conceptualised as containment and mitigation and focuses on actions to eradicate the pest, halt its spread and reduce its impacts. For many actors this stage forms the core of what is commonly perceived as 'outbreak management' and includes a great deal of the focused action undertaken by governmental bodies and others. Clearly, the boundary between this and the preceding stage is highly contingent and will vary substantially between outbreaks. Indeed, to a certain extent the two stages necessarily run contemporaneously, with perhaps the primary distinction being that relevant human activities are mainly unintentional during the spread stage, and more directed and active in the containment and mitigation stage. Given its complexity and significance in terms of active management of pests, it may be useful in some cases to sub-divide this stage into different phases. There are potentially a number of sub-phases including eradication, local containment, landscape scale management, and then mitigation. Decision to move between these phases may have profound consequences for the resources that can be mobilised and the range of stakeholders engaged in outbreak management.

When and where management has failed to contain the spread of a pest, or has succeeded only in reducing its impacts, a final adaptation stage can be identified wherein stakeholders have to learn to live with a pest's presence and impacts. During this stage some profound changes to problem definitions and significant changes to management practices are highly likely as adaption to living with the pest or disease entails different levels of engagement in tree health by a number of stakeholders with the aim of coming to terms with loss and promoting future resilience.

This stage-based model of stakeholdership does not necessarily develop in a linear fashion. Whilst biological models of invasion can to some extent be represented chronologically, boundaries between the socially defined stages in this framework can be relatively fluid. It is possible for some stages to occur or reoccur at different points of an outbreak, depending on the social factors involved. For example, a tree pest or disease could be defined as a problem prior to introduction (i.e. at the pre-pathway stage) via a pest risk analysis, or it may not be defined as a problem until the post-introduction stage, once impacts begin to be recognised. Furthermore, different stakeholders can be located within different stages of the same outbreak at the same moment in time, depending on how they engage with it; for example, some stakeholders may be working on adaptive strategies, whilst others may still be focused on mitigation or containment[1]. This highlights the intrinsic dynamism, or 'messiness', of outbreaks.

Active, passive or latent stakeholders

Alongside the development of an outbreak over time, changes in stakeholdership brought about by varying levels of knowledge, awareness and certainty relating to tree pests and diseases can also have a major influence on the make-up of the tree health stakeholder landscape. In essence, whilst a *de facto* stake may exist for particular individuals and groups, a lack of awareness, knowledge or certainty as to the character and extent of that stake deeply influences those stakeholders' participation in tree health management. Differences in awareness can be the result of numerous factors or events such as direct encounters with the pests or pest management activities, media coverage, or informational campaigns. This generates a particular stake for some – especially media and other actors who have a stake as networkers. These actors have the capacity to make people aware of their stakes and thus enrol them into prevention, containment or adaptation efforts.

At a basic level, a divide can be made between stakeholders who have recognised a stake (even if not necessarily fully or correctly) and those who have not. Those who have recognised their stake in tree health can be classed as either active or passive stakeholders. Active stakeholders have realised their stake and are actively engaged in the outbreak - at least in terms of knowledge gathering or monitoring events. Passive stakeholders have also realised their stake, but have made a choice not to engage, perhaps on the basis of judgements about vulnerability, responsibility, or a lack of resources. This might include householders and businesses who stand to bear significant, uncompensated costs for eradication or containment measures such as felling and chemical treatments, or woodland owners who may be aware of the threat to their trees, but who judge the investment of time and resources in good biosecurity not to be worthwhile. Those who have not recognised their stake in tree health can be labelled as unrealised (or latent) stakeholders and are very unlikely to be engaged with the outbreak. Local residents, who may be vulnerable to an outbreak but have no knowledge of it fall into this category. Others may include those with a potential networking stake but are not engaged because their stake is less direct and not immediately connected to tree health.

3. Case-study: Who has a stake in ash dieback?

In this case-study we focus on the ash dieback outbreak in the UK, drawing on the conceptual framework to categorise the stakeholders involved. We trace when different stakeholders became engaged in the outbreak and identify their role as influencers and stakeholders of interest. We then reflect on the usefulness of our framework as a critical tool for stakeholder engagement.

3.1. Outbreak timeline

Pre-pathway and mobilisation: Early warning signs and emergence in the UK

Ash dieback was first identified in Europe in Poland in 1992 (Kowalski 2006). Once established there, it was mobilised onto the pathway and subsequently spread to about 25 European countries over the following decades. Prior to 2012, there was no systematic surveillance in the UK for ash dieback beyond general monitoring for general tree health, assessments for the disease during the National Forest Inventory (NFI), and the UK Plant Health Service's general pest and disease surveillance programme (Sansford, 2013). Although the FC had discussed whether an import ban might be appropriate with the European Commission's Plant Health Standing Committee (PHSC), confusion about the identification of the pathogen made this problematic.

Ash dieback was first identified at a nursery in Buckinghamshire amongst 600 trees from the Netherlands during a routine inspection in February 2012 by the Food and Environment Research Agency (FERA) By August of the same year, ash dieback had been confirmed at four other nursery sites in England. A Rapid Pest Risk Analysis

---

[1] Scale can be particularly influential here as adaptation may, for example, be the goal of national stakeholders and directly affected stakeholders, whilst those at local levels and (as yet) unaffected by the pest or disease may be focused on avoiding spread.

by Forest Research (Webber and Hendry, 2012) in August 2012 found a range of sites of infection, including newly planted amenity woods and nursery stock, but also extending into the wider environment, especially in eastern and south-eastern England and Scotland.

Introduction, establishment and spread: The ash dieback 'crisis'

In October 2012 the situation escalated with intense media coverage and public concern over the disease. Concern focused on the potential loss of a beloved British tree and the impact on wider biodiversity. At the same time, the disease was attracting political attention and was seen as a 'national crisis' (HCDeb, 2012). A Plant Health Order was issued in October 2012, placing restrictions on importing ash trees into Britain (FC, 2012) which effectively meant a ban on all movements of ash plants or seeds within Britain. Few in the wider forest sector were aware of the extent of the plant trade, including the high number of UK ash seedlings that were being sent to the continent for growing, which is otherwise known as 'ex-importing' (Brasier 2008) A survey by the Horticultural Trades Association (HTA) estimated that around 4 million ash trees had been imported between 2009-2012.

The week after a Plant Health Order was issued, the UK Government's emergency 'Cobra' committee met to discuss how to deal with the outbreak. A rapid survey over the following weekend of a thousand sites that had been planted with ash saplings from infected nurseries was undertaken. Alongside this, a Tree Health and Plant Biosecurity Expert Taskforce was set up to provide independent multi-disciplinary academic expertise, develop strategy and assess the current disease threats to plant health more broadly.

In December 2012, the UK Government published an 'Interim Chalara Control Plan' that outlined a strategy for reducing the spread of ash dieback, developing tolerance in the UK's native ash tree population, and encouraging public and industry action to tackle the problem. Part of the plan involved providing funding for the continued development of citizen science programmes and several ash dieback-specific research projects.

Kent and East Anglia were the first areas in England to be badly affected by ash dieback. In East Kent there have been hot spots of severe infection since 2012, with both young and mature ash being killed. This has led to extensive landscape change in some areas. Kent County Council and other partners declared the outbreak a 'major incident' in November 2012 under the Civil Contingencies Act (2004). As a result, the council set up a multi-agency Strategic Co-ordinating Group (SCG). Partners included county and borough councils, Department of Communities and Local Government, Environment Agency, Kent Tree Officers Group, the Highways Agency, Kent Police, Forest Research and the UK Power Networks. A key concern was the potential financial costs of the outbreak in terms of effective monitoring and timely outbreak management actions (SCG, 2012).

A Pest Risk Analysis published in 2013 identified four main pathways of entry for ash dieback into the UK: the live plant trade, wood, seeds, and contaminated soil (both as a commodity, or with host or non-host plants) (Sansford, 2013). It is believed that the importation of infected plants is the main route of entry into the UK (Sansford, 2013). In addition, meteorological modelling by the University of Cambridge suggested that airborne incursion from the continent was also likely and explained the appearance of ash dieback in natural woodland sites (Defra, 2013), although these findings are contested (Chandelier et al., 2014, Mabbett, 2014).

Containment and mitigation: 'Slowing the spread'

The enrolment of stakeholders in this stage is largely contingent on the geographical spread of the disease. For those dealing with ash dieback in Kent or East Anglia, where the impacts have been the most acute to date, the focus is on containing the disease to slow the spread into uninfected areas, as well as mitigation activities such as removing or pruning diseased trees where there is a public safety risk, and building resilience in existing woodlands. Some stakeholders in these areas, such as public stakeholders, landowners and local authorities, may be moving into the 'adaptation' stage where they recognise that ash dieback cannot be eradicated and there is a need to 'learn to live with it'. Indeed, the government's early recognition that it would not be possible to eradicate ash dieback led to strategies that focused on containment and mitigation, while adaptation measures (such as identifying tolerant strains of ash and building resilience in woodlands) were developed. This is reflected in documents such as the Tree Health Management Plan published by Defra (Defra, 2014).

3.2 Applying the conceptual framework to ash dieback

Through an analysis of the ash dieback case, we have identified a broad range of government, private, civil society and other actors with a stake in ash dieback (Table 2).

Table 2: Stakeholder categories for the ash dieback case study

	Private Sector	*Governmental*	*Civil Society*
	INFLUENCE		
Governors	ConFor	Forestry Commission EPPO EU PHSC DEFRA WTO UK Border Agency Chief Plant Health Officer	
Vectors	Plant nurseries Private land managers Outdoor recreationists Forestry & Arb. Contractors	Forest Enterprise Local Authorities	Land managing organisations (National Trust; Wildlife Trust; Woodland Trust)
Managers	Forestry & Arb. Contractors Forest owners & managers	Forestry Commission Forest Research Local Authorities UKBA FERA SCG in Kent	National Trust Wildlife Trusts Woodland Trust
Monitors	Forestry / Arb. Contractors Environmental consultants Forest owners Citizen scientists General public	Forest Research Forestry Commission FERA Universities & research institutes European plant pathologists Tree Health & Plant Biosecurity Taskforce Norfolk County Council Kent County Council EU projects	Woodland Trust Suffolk Wildlife Trust
Networkers	ConFor CLA HTA Local & national media	Forestry Commission EPPO	ICF National Trust Woodland Trust RHS
	INTEREST		
Outcome Winners	Forestry / Arboricultural Contractors Environmental consultants Scientists	Forest Research Universities & research institutes	
Value Loser	Homeowners / Landowners Forest owners Local residents & communities Recreationists	Forest Enterprise	CPRE National Trust Wildlife Trust Woodland Trust
Cost Loser	Homeowners Forest owners Plant nurseries Plant traders & importers	DEFRA Forestry Commission Local authorities	Land managing organisations (e.g. National Trust Wildlife Trust Woodland Trust)

Table 2 (continuation)

Collateral Loser	Plant nurseries	DEFRA Forestry Commission High Weald AONB MPs & political parties	Woodland Trust National Trust
Contributors	Plant nurseries & buyers Outdoor recreationists Landscape planners Forest managers/owners	Forest Enterprise Forestry Commission	Woodland Trust National Trust

Categories of influence
Government bodies that include national government departments, EPPO and the World Trade Organisation are the main *governors* in the ash dieback case study. During the pre-pathway stage, UK government bodies such as Defra, the Forestry Commission and the UK Border Agency were responsible for ensuring biosecurity of the live plant trade and undertaking surveillance for pests and diseases not yet present in the UK. However, there were particular difficulties faced by these stakeholders, including the early confusion over the identification and nomenclature of the pathogen which made it especially difficult for the government to regulate. Eventually, once ash dieback was introduced to the UK and received the associated public and media attention, Defra was able to issue a Plant Health Order to ban imports and movement of ash in the UK, to commission the Tree Health and Plant Biosecurity Taskforce, and to undertake the national survey of the disease and provide funding for scientific research. Strong links between governors and monitors are evident here.

The largest group of *vectors* were private businesses, such as tree nurseries, involved in the importation of ash saplings. This trade also involved government bodies such as Forest Enterprise and civil society organisations, such as the Woodland Trust and National Trust. As the outbreak developed, further vectors for spread included forestry and arboricultural contractors dealing with infected trees. Outdoor recreationists were also potential vectors, although this pathway is less likely.

Government agencies and local authorities also had a lead role as '*managers'* of the outbreak , through surveillance and by engaging in control and treatment of infected trees. Organisations such as the Highways Agency and Network Rail were also important managers, as ash is a common tree on roadside verges and railway embankments and these organisations had the skills and responsibility to both monitor and care for them. In addition, the UK Border Agency is responsible for managing infected plants at the border. Private forestry and arboricultural contractors, as well as forest managers, were also engaged in directly undertaking works on infected trees, or, in the early phases of the outbreak, removing newly planted infected ash saplings.

Key *monitors* during the pre-pathway stage were plant pathologists across Europe who were trying to identify the pathogen responsible for the disease. During the early phase of the outbreak, the Tree Health and Plant Biosecurity Taskforce had an important monitor stake through its assessment of the government's approach and recommendations for a future management strategy. As the outbreak unfolded further, scientists, universities and research institutions were enrolled in the outbreak in order to: identify pathways of introduction (including potential for natural windborne spread), improve detection methods, identify tolerant strains of ash, and explore the potential societal impacts and public risk perceptions associated with the disease. Community and public groups were also engaged with, first by early calls from the Forestry Commission urging them to be on the lookout for signs of disease, and then through a number of citizen science programmes aimed at enrolling the public in wider early detection, monitoring, and networking.

The '*networkers'* group of stakeholders is particularly interesting in the ash dieback case. The national media has played a significant role in communicating about the emergence and spread of the disease, with many of the stories calling into question the government's competence to deal with breaches in biosecurity. The intense media focus in late 2012 catapulted ash dieback into the public spotlight and as a result spurred government action. Some civil society organisations, such as the CLA and the Woodland Trust, were vocal in expressing their concerns about the disease, and one of the early 'networkers' was the HTA, who wrote to the FC in 2009 expressing concern about a disease of ash that their members had witnessed in Denmark. Private nursery businesses also express their concern about the disease through the Nursery Group of ConFor. Kent County Council's action to bring together a wide range of government, private and civil society stakeholders in order to coordinate management of the disease in the county is a rare example of a networker playing a more positive role.

Categories of interest
Value losers included residents affected by landscape change, biodiversity loss and recreational impacts (either through degraded recreational space or closed sites). Other value losers include private, public and NGO woodland and tree owners, both in terms of negative impacts on stock, and to ecosystem services (e.g. timber, woodfuel). However, perhaps the most numerous value losers in this were members of the public who saw significant potential to lose a much loved and valued British tree.

Cost losers in the outbreak were plant nurseries that had to destroy large quantities of infected ash sapling stock, as well as woodland owners who have been and will be financially affected by the costs of undesired or early felling, and of restocking woodland. Local authorities in affected areas undertake the cost of funding public safety arboriculture work on diseased or dead trees in their areas, which is likely to increase over time as the disease spreads and further local authority stakeholders become enrolled. Another major cost loser is central government through funding management of the disease and funding scientific research: although some of this resource went to other government bodies increasing their resources

Perhaps the most prominent *collateral loser* is the UK government, specifically in regard to their loss of reputation. Much of the early public and media scrutiny criticised the government's handling of the outbreak, and it could be argued that many subsequent government actions have been blame avoidance strategies to reduce risks to reputation. Further, private plant nursery businesses also face risks to their reputation, due to their role as 'vectors' of the disease.

The ash dieback case highlights a wide-range of *contributors* across government, private and civil society domains. This reveals the difficulty of managing a plant disease that is embedded in the global trade in live plants. Public, private and NGO forest managers seeking stock at the best price created a market for the trade in live ash trees. Growers, suppliers, traders and customers all benefit from this demand, but their activities are complicit in the introduction and spread of the disease.

Arboricultural and forestry contractors are clear *outcome winners*, at least in the short term, as the emergence of the disease provided increased sources of income in dealing with affected ash trees. Further financial benefits accrue for environmental consultants, scientists, universities and research institutions who have also benefited from increased funding for work on ash dieback and tree health more broadly.

4. Conclusion and Policy Implications

This brief analysis of the ash dieback case study has enabled us to sort and classify the stakeholders currently involved in tree health. It also provides insight into how stakeholdership changes over the course of an outbreak. Very few outcome winners were identified, but there were a large number of government, private and civil society losers. The 'vectors' and 'contributors' were largely those businesses associated with the live plant trade, along with their customers. However, those implicated in the introduction and spread of ash dieback have also often been 'cost' and 'collateral' losers. A broad range of governmental bodies have been identified, including several (such as transport bodies) that have seemingly limited involvement with tree health. The media and some private and civil society institutions also emerge as networkers.

We are also able to reflect more critically on tree stakeholder engagement through the framework's lens, and learn lessons for the future. For instance, it is useful to make explicit links between the strong behaviours exhibited by monitors and governors. In the ash dieback case, scientific uncertainty fed inertia amongst policy-makers who did not act on less formal observational 'evidence' of the threat. There are, however, some encouraging signs understanding amongst monitors is improving in the wake of ash dieback, as this category has proactively expanded through engagement initiatives like citizen science. However, governors need to ensure that appropriate processes are in place to effectively take advantage of information that originates from these new sources.

Various arms of the UK government are identified in the ash dieback case, each with their own priorities and stakes in a tree health outbreak situation, but criticism was often directed at 'government' as a whole. Arguably, this case illustrates the political power of public opinion because much action was instigated by the widespread anger at the thought of losing an iconic British tree species. This is similar to what happened with the occurrence of Dutch Elm disease in the UK in the 1970s and '80s.

Due to the nature and evolution of the ash dieback case in the UK, stakeholder engagement has been primarily focused on managing the outbreak itself – that is, on the spread and containment stages. Critically, there is little evidence in this case of pre-pathway engagement, despite knowledge that the threat of ash dieback existed. Furthermore, positive engagement with networkers was virtually absent throughout the outbreak. An understanding of the role these networker stakeholders can play *should* incite their early engagement. In the case of ash dieback, networkers focussed on a reactive critique of the situation, without acknowledging that they could have taken a more productive role in raising awareness. In the case of ash dieback, plant nurseries are 'contributors' and 'vectors', but also 'cost' and 'collateral' losers. Improving understanding about the balance between potential costs and benefits amongst this group seems particularly important. Networker stakeholders have a key role in facilitating such learning and might employ a range of regulatory and market mechanisms, along with strategic communication and education activities.

The retrospective application of our conceptual framework has allowed us to categorise who has which stake in tree health. It has also helped us to reflect on the tree health stakeholder landscape more broadly and critically to identify possible interactions between different categories of stakeholders. Acknowledging these dynamics can lead to improved interventions to better prevent or control future tree disease outbreaks. The tree health sector, and forestry sector more widely, has begun to take stakeholder analysis and engagement seriously: but this has brought significant challenges. Particularly challenging is identifying which actors have important stakes, when and, critically, who should engage them and how? The tree health stakeholder landscape is broad and complex. If stakeholder analysis is too narrow, subsequent engagement will have only limited impact on the outcomes of pests and diseases. If it is too broad, engagement will be too difficult and costly a job for whichever organisations take it on. The framework described here should allow those charged with responding to the threats of tree pests and diseases to rationalise and manage their engagement: targeting it towards the right stakeholders at the right times. It can also facilitate a more holistic and efficient view which takes in multiple pest and disease threats, instead of continuously focusing efforts on individual organisms.

Acknowledgements

This research was undertaken as part of two projects: *Mapping, Analysis and Improved Understanding of Stakeholders and the Public To Help Protect Tree Health* (Project TH0104) and *Understanding Public Risk Concerns: An investigation into the social perception, interpretation and communication of tree health risks* (BB/L012308/1), which were both funded under the Tree Health and Plant Biosecurity Initiative in the UK.

References

Brasier, C.M. 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathology, 57, 5, 792-808.

Chandelier, A., Helson, M., Dvorak, M. & Gischer, F. 2014. Detection and quantification of airborne inoculum of *Hymenoscyphus pseudoalbidus* using real-time PCR assays. *Plant Pathology*, 1-10.

Colautti, R. I. & MacIsaac, H. J. (2004) A neutral terminology to define 'invasive' species. *Diversity and Distributions* 10, 143-146.

DEFRA and FC. 2011. Tree Health and Plant Biosecurity Action Plan. Department for Environment, Food & Rural Affairs

DEFRA 2012. Interim Chalara Control Plan. Department for Environment, Food & Rural Affairs.

DEFRA 2013. Chalara Management Plan. Department for Environment, Food & Rural Affairs.

DEFRA 2014. Tree Health Management Plan. Department for Food, Environment and Rural Affairs.

FC 2012. The Plant Health (Forestry) (Amendment) Order 2012. Forestry Commission.

Freeman, R.E., 1984. Strategic Management: a Stakeholder Approach. Basic Books,New York.

Friedman, A.L. and S. Miles (2006) Stakeholders: theory and practice. Oxford: Oxford University Press

Gilmour, J., R. Beilin, T. Sysak (2011) Biosecurity risk and peri-urban landholders–using a stakeholder consultative approach to build a risk communication strategy. Journal of Risk Research,

HCDEB 2012. Hansard Debates 12 November, col 84. House of Commons: Hansard.

Heger, T. & Trepl, L. (2003) Predicting Biological Invasions. *Biological Invasions,* 5, 313-321.

Hernández-Jovera, M., J. Gilmour, N. Schembri, T. Sysak, P.K. Holyoake, R. Beilin, J.-A.L.M.L. Toribio (2012) Use of stakeholder analysis to inform risk communication and extension strategies for improved biosecurity amongst small-scale pig producers. *Preventive Veterinary Medicine* 104, 258– 270

Heuch, J. 2014 What lessons need to be learnt from the outbreak of Ash Dieback disease, *Chalara fraxinea* in the United Kingdom? Arboricultural Journal, 36, 32-44.

Kowalski, T. 2006. Chalara fraxinea sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland. *Forest Pathology,* 264-270.

Lienert, J., F. Schnetzer, K. Ingold (2013) Stakeholder analysis combined with social network analysis provides fine-grained insights into water infrastructure planning processes. *Journal of Environmental Management* 125: 134-148.

Mabbett, T. 2014. Ash die back: The latest on Chalara. *International Pest Control Magazine*.

Maguire, B., J. Potts, S. Fletcher (2012) The role of stakeholders in the marine planning process—Stakeholder analysis within the Solent, United Kingdom, *Marine Policy*, 36, 246–257

Mills, P., Katharina Dehnen-Schmutz, Brian Ilbery, Mike Jeger, Glyn Jones, Ruth Little, Alan MacLeod, Steve Parker, Marco Pautasso, Stephane Pietravalle and Damian Maye (2011) Integrating natural and social science perspectives on plant disease risk, management and policy formulation. Phil. Trans. R. Soc. B. 366, 2035-2044

Porth, E., Marzano, M. & Dandy, N. (2015) "My garden is the one with no trees": Residential lived experiences of the 2012 Asian longhorn beetle eradication programme in Kent, England., *Human Ecology*

Prell, C. K. Hubacek, M. Reed (2009) Stakeholder Analysis and Social Network Analysis in Natural Resource Management. Society and Natural Resources, 22:501–518

Reed, M. S., A. Graves, N. Dandy, H. Posthumus, K. Hubacek, J. Morris, C. Prell, C.H. Quinn, and L.C. Stringer (2009) 'Who's in and why? A typology of stakeholder analysis methods for natural resource management', *Journal of Environmental Management*, 90, pp. 1933-49.

Sandford, C. E. 2013. Pest Risk Analysis for *Hymenoscyphus pseudoalbidus*. Forestry Commission.

SCG 2012. Minutes of Meeting of The Kent Resilience Forum Ash Dieback Strategic Co-ordination Group (SCG).

Sandstrom, C., A. Carlsson-Kanyama, K. B. Lindahl, K. M. Sonnek, A. Mossing, A. Nordin, E Nordstro¨m, R. Raty (2016) Understanding consistencies and gaps between desired forest futures: An analysis of visions from stakeholder groups in Sweden, Ambio, 45(Suppl. 2) S100–S108

Stoll-Kleemann, S., M Welp (2006) Towards a more effective and democratic natural resources management. Stakeholder dialogues in Natural Resources. Springer Publishing.

Tomlinson, I., C. Potter and H. Bayliss 2015 Managing tree pests and diseases in urban settings: The case of Oak Processionary Moth in London, 2006–2012, Urban Forestry and Urban Greening, 14, 286-292.

Van de Kerkhof, M. (2004). Debating climate change. A study of stakeholder participation in an integrated assessment of long term climate policy in the Netherlands. Utrecht: Lemma Publishing.

Webber, J. & Hendry, S. 2012. Rapid assessment of the need for a detailed Pest Risk Analysis for Chalara fraxinea. Alice Holt: Forest Research

Williamson, M. (2006) Explaining and predicting the success of invading species at different stages of invasion. *Biological Invasions,* 8, 1561–1568.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 27 – 32.

Socio-economic outcomes of ash die-back in Romania

M. DRĂGOI*[1], D. CHIRA[2], O. POPOVICI[3]

[1] Faculty of Forestry, University of Suceava, 720229 Suceava, Romania, [2] National Institue of Forest Research and Development in Forestry "Marin Dracea", 077190 Voluntari, Romania, [3] Suceava County Office, National Forest Administration ROMSILVA, Suceava, Romania.
* corresponding author: marian.dragoi@usm.ro

Abstract

Ash dieback is a complex and challenging issue most of European countries have to cope with. In addition to the technical and biological aspects of the problem the socio-economic impact is another important subject, given that common Ash (*Fraxinus excelsior*) is an important component of broad-leaved mixed forests event though its share at national scale is very small. Its status stems from the quality of the wood which, in spite of its low availability, has been used in rural communities for centuries and nowadays is sought by veneer producers and high-quality wood-based sport equipment. Because neither the forest managers, nor the forest planners and the environmentalists have got the adequate knowledge to cope with the ash dieback and the current state-of-the-art has failed to find out a certain course of preventive actions, a field study was carried out in order to figure out the needed changes of foresters' behavior and attitudes with respect to rapid die-off of rare but valuable forest species, like the common ash. The study was based on the theory of planned behavior and its main goal was to design an awareness campaign among foresters considering that protective measures needed to slow down the ash dieback as long as they have to come up with a completely different set of protective measures, stemmed on new attitudes.

Therefore, a socio-economic survey was carried out among foresters and other important stakeholders in order to find out 1) to which extent the ash dieback is perceived as important and for what reasons, in wood industry and rural areas, 2) whether or not the current management practices may contribute to preventing the propagation of ash dieback and 3) which set of actions must be urgently undertaken in order to prevent disease propagation according to the general perception shared by lay people and foresters with respect to the ash dieback. The results are summarized as follows: 1) most of the forest professional do not fully realize the potential risk of having the ash trees harvested as salvage wood when it comes to the commercial and economic impact of ash dieback 2) most of respondents consider that more seedlings produced in nurseries is a reliable solution for having the problem solved on long-term, 3) an important share of foresters pinpoint the logging damages as the main causes of ash dieback, and 4) an insignificant number of respondents are fully aware of the new challenges raised by ash dieback when it comes to the new procedures deployed to thin the younger stands where bunches of ash tree are to be found, in broadleaved mixed forests.

In addition to these main conclusions, it was found that the expected ecological value of common ash is not fully recognized by a great deal of forest-related stakeholders, mainly because the amount of valuable wood is very small at the national scale and the rate of marginal substitution of ash wood with other species is quite high. This conclusion does not apply when it comes to the common utilizations of ash wood in rural areas, where traditional woodcraft activities are seriously jeopardized by ash die-off.

Introduction

Although ash is not one of the most prevailing forest species in Romania, its ecological and economic importance cannot be overlooked. After elm die-off, which has occurred by late nineties, another important pillar of broadleaved forest ecosystem is being threatened to extinction and makes room for a series of debates on the technical possibilities to replace common ash with another species of ash, likely to be shortlisted as an invasive species, or to change the forest management in order to cope with this new challenge.

The symptoms of ash die-off develop rapidly and the perspective of having harvested all ash trees is more and more evident if we contemplate similar processes that took place in Belgium (Chandelier, Delhaye et al. 2011), Austria

(Kirisits, Matlakova et al. 2009), Estonia (Lõhmus and Runnel 2014), Great Britain (Mitchell, Beaton et al. 2014), or Italy (Ogris, Hauptman et al. 2010).

The technical challenges raised by the ash dieback are complex and involve a series of actions to be undertaken at stand level by different stakeholders, like forest planners, professionals responsible with timber cruising and pest control. Equally important are harvesting procedures, deployed now according to the technical standards. Because dead trees are spread out on large areas, the break-even point from harvester's stand point is reached only hauling a few valuable trees and, in spite of high economic value of ash wood a great deal of dead trees may have remained unharvested in due time into the forests, and the risk of die-off may be even higher. Therefore, the specific management of ash dieback has a quite challenging statute, being somewhere between the crises management (Jaques, 2004) and the change management (Todnem By 2005). For forestry such situations were quite unusual a decade ago, when most of all aspects of forestry were highly predictable and pursuing the provisions of the management planning system was not a problem.

As (Ajzen, 2011) has noted, the TPB list of reference has grown exponentially in the last 20 year, according to Google scholar reference system. The decomposed version of theory of planned behavior was used to study the intention to pay for different services available in the virtual community (Horng, Shwu-Min, Yih-Yuh Lee, 2012)

Material and method

The die-off a tree species like common ash, which is not representative for the main types of forest ecosystems but still is valuable from the economic point of view, might be unimportant for a logging companies, alarming for wood crafting and critical for a biologist. Forest managers, forest planners, plain people are facing an over-supply of ash wood and this issue might have a social dimension, rendered by beliefs and attitudes. Therefore we have tried to adapt the theory of planned behavior to our problem in order to evaluate to which extent different stakeholders might be focused on finding a new technological solution to ash die-back or, on the contrary, people will be interested in taking advantage of this situation by harvesting more and more ash trees, without any concern for the future.

Theory of Perceived Behavior (TPB) allows predicting individual behaviors and it is widely used to explore the determinants of professional behavior. The theoretical main assumption the TPB relies on is that an individual's intention to pursue an expected course of actions (i.e. behavior) is predicted by her or his attitude, assuming that attitude itself is an overall evaluation of the behavior

.

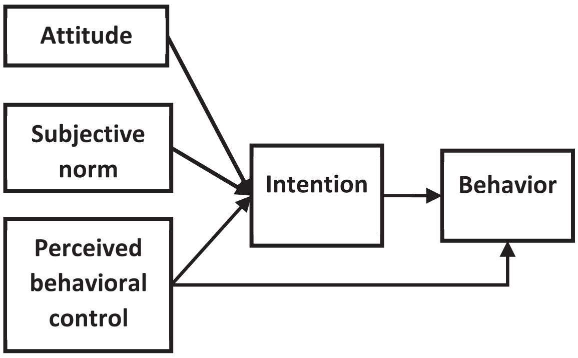


Figure 1 Theory of perceived behavior (a person's overall evaluation of the behavior), subjective norm (a person's own estimate of the social pressure to perform or not perform the target behavior), and perceived behavioral control.

The PBC refers to individuals' perception of the ease or difficulty of performing the behavior of interest (Ajzen 1991). In case of ash dieback, the individuals who have been questioned have been facing a new situation in which they lack complete volitional control over the behavior of interest, meaning that they had to choose a series of answers to a series of closed questions they were not familiar with, simply because the main topic of the survey is a widespread die-off process, they never have encountered so far. Molding the die-off perception into the PBC shape we have assumed, according to the theory of planned behavior, that the biodiversity value of common ash should influence behavior indirectly by guiding a person's beliefs and attitudes in order to apply in situ conservation techniques, if that person would be a professional forester dealing with timber sales. Another type of action would be ex-situ conservation, which covers a larger spectrum of actions, from producing more seedlings of common ash to ash interbreeding. One objective of the investigation, therefore, was to examine the extent to which the theory affords prediction of appropriate conservation measures, and whether the effect of values on behavior is indeed mediated by the theory's more immediate determinants (attitudes, subjective norms, perceptions of control, and intentions) will produce a certain outcome.

The study is based on a survey delivered to a series of stakeholders involved in different levels of forest management, more or less directly affected by ash die-off, in order to find out some relevant information about: 1) the public perception about the biodiversity loss produced by ash die-back; 2) the economic loss on long run, assuming that all ash trees would have disappeared from Romanian forests; 3) the possible management scenarios that might slow down the dieback process; 4) foresters' perception on possible scenarios meant to stop the dieback and 5) foresters' opinion about a new breading programs involving different ash species.

The survey was structured as follows:
Section A. General information about the surveyed person and s/his professional profile with respect to the forest species die-off in general (forest manager, conservationist, forest planner, logger or whatever timber end-user).

Section B. How to manage the die-off of European ash? How often the interviewee encountered ash die-off, personal evaluation ash trees life expectations on different. An intriguing closed question refers to the possible management alternatives worth being considered in order to save the European ash from dying-off. This section is important because all feasible actions were considered and it aims at assessing the technical acceptability of some complex measures foreseen by foresters, based on the ecology of *Chalara* sp.

Section C is focused on highlighting the commercial value common ash. It is based on a Likert scale and the following issues have been addressed: the use value of ash timber, the expectations for having a much higher value on the future, the marginal rate of technical substitution for ash wood, as well as the technical possibilities to replace the common ash with more resistant hybrids, having in mind that a serious die-off might increase the demand for different hybrids between ash and manna or might encourage new breeding programs.

Section D addresses the social impact and economic impact of having this species lost, having in mind the common utilization of ash wood, which is sought after for lumber, furniture, parquet, interior paneling, woodcraft and even traditional rural utilization, including carriages.

Section E addresses the perspective on the ecological roles played by common ash at landscape level, habitat conservation and biodiversity.

The survey was structured in order to allow apprising 1) the degree to which each main group of stakeholders makes use of the information at hand about *Chalara* in a rational way and 2) how people are aware of the outcomes of their actions and, according to those outcomes, they get involved in a given course of action or behavior. The salient assumption we have made is that most of the stakeholders are not fully aware of connection between their endeavors and the health of the forest they harvest the timber from.

Results

The survey was distributed to 350 foresters, forest loggers and specialists from GNOs in printed copies and over the Internet. The ecological impact, as shown in Figure 2 is quite unexpected because a lot of respondents did not see a serious problem from the ecological point of view because they did not consider that a high commercial value may be a threat for the remnant ash trees, which somehow makes sense. More significant agreements have been found at rural level, because people are expecting to have access to lesser amounts of durable wood for wood-crafting and hafts, which are important utilizations of common ash wood.

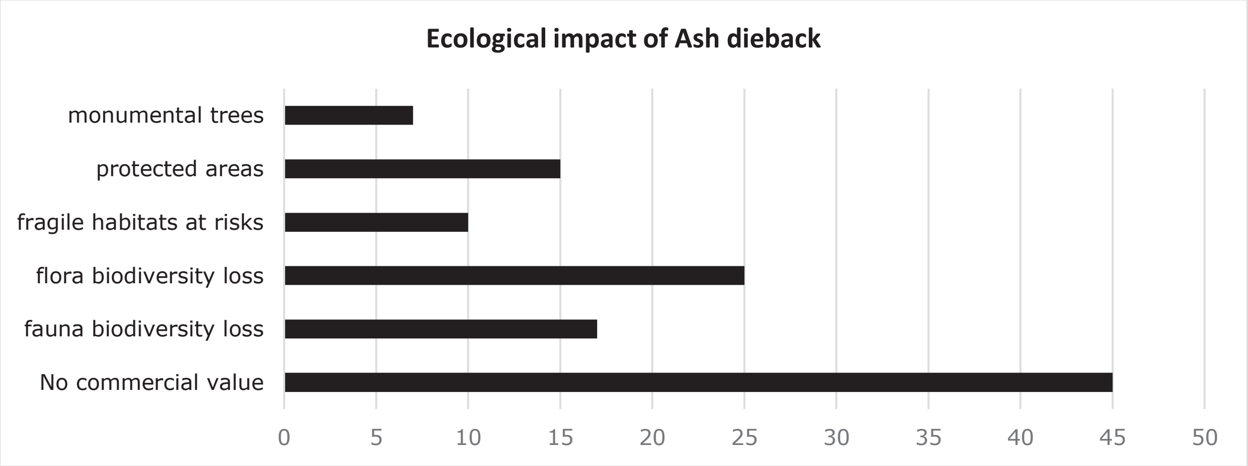


Figure 2 Ecological impact of ash dieback

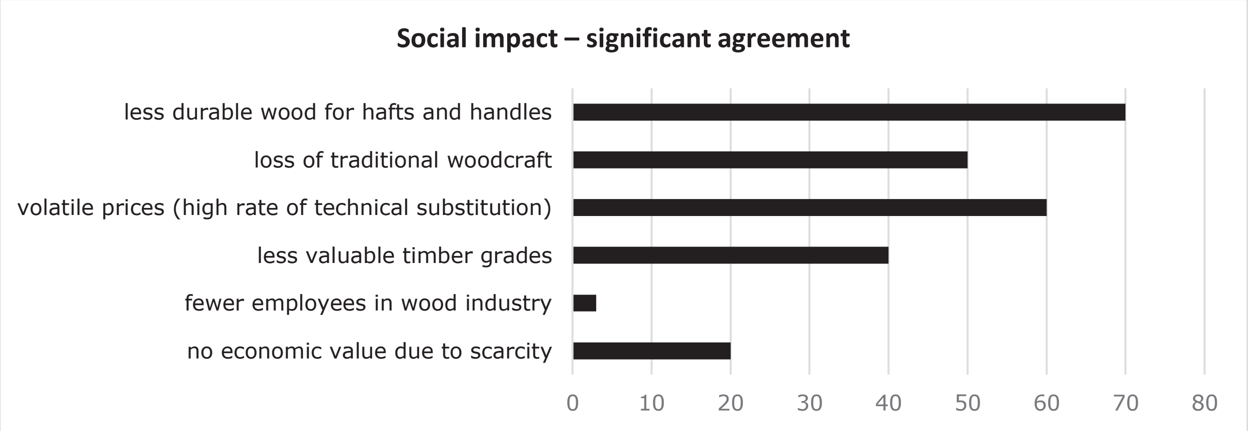


Figure 3 Social impact of ash dieback

The survey tried to figure out to which extent foresters and plain people as well are aware of the connection between climate change and ash dieback. High frequency of ash dieback has been brought about by lack of adaptive management, which could be seen also as a sort of red herring, because all bad things happening in the forest might be caused by lack of adaptive management. When it comes to the degree to which fungi or insects' occurrence is connected to climate change, most of the respondents considered that insects are more threatening, as shown in Figure 4, where the y axis represent the intensity of the connection (importance) between climate change, insect attack and fungi propagation.

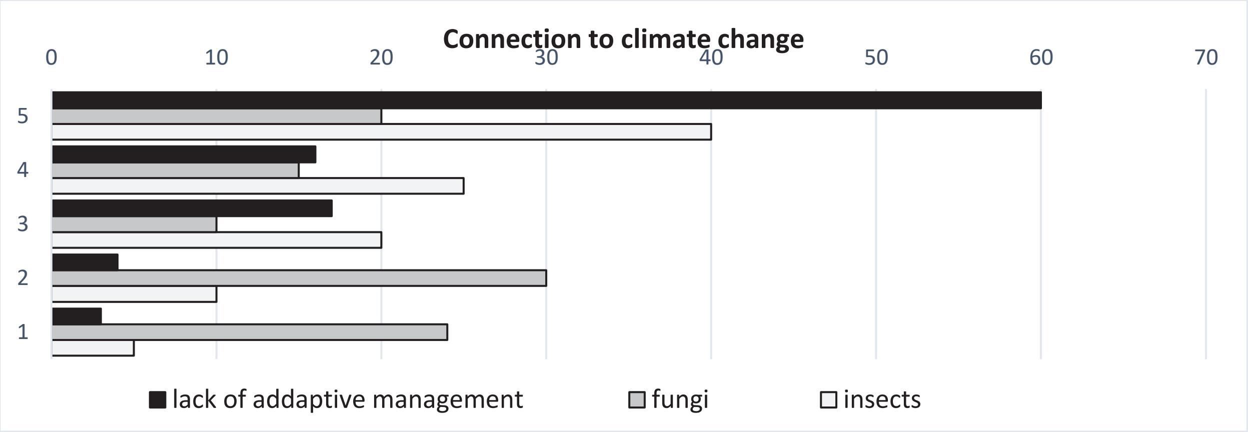


Figure 4 Connection between ash dieback and climate change

Actions foreseen to eradicate or reduce ash dieback

The survey has offered eleven options to control the die-off process, as follows:

- more seedlings produced in nurseries, in order to replace dead trees with the new ones after cuttings
- to avoid plantations with ash in affected areas
- to replace common ash with hybrids
- to adopt special conservation measure for the ash trees that survived, in order to reproduce them in nurseries
- to avoid logging damages to remnant trees
- to adopt special conservation measures during harvesting operations
- to let more light reaching the soil in order to dry out ash litter
- to remove the dead trees as soon as possible
- fertilization measures in new plantations
- better control against insects
- better control against fungi by chemical means, where available and affordable

The contingency table of these options is presented in Table 1. Because more options were allowed, the sum of the occurrences is greater than 350, which is the total number of respondents.

Table 1 Contingency table of the combinations of preventive measures chosen by interviewees.

	1	2	3	4	5	6	7	8	9	10	11
1		40		10	10	30	40	60	3	30	5
2				30	78	40		30			
3					39	35	34	6		34	10
4						56	30		1		
5										45	
6								45	3		
7								23		12	
8									3		
9											3
10											
11											

One may notice that most of the people who replied to the survey are prone to seek the solution into a narrow area, implying more caution during harvesting operations, greater amounts of seedlings produced in nurseries in order to replace the affected trees and better control against insects, combined with new ash hybrids replacing the common ash. An important outcome, reflecting a better knowledge of the way in which *Chalara* propagate is the high rate of respondents who had seen a possible solution in heavy thinnings nearby the ash trees, in order to deter the fungi propagation through the ash litter. Nevertheless, this option is challenging and provocative for the technical standards applied now in Romania, because it implies a completely new approach of forest management at stand level.

Discussion and conclusions

Because the study was localized in the north of Romania, just in two counties (Suceava, and Botosani), the result are not conclusive at national level but they reflect the fact that excepting the professional foresters quite a few other stakeholders are aware of and concerned about the ash dieback consequences and severity. Nevertheless, plain people from rural areas are more concerned compared to other stakeholders because they associate the ash wood with a large variety of tools they have been using in their households. Apparently the less concerned are the forest professional who are working in harvesting operations, because the ash wood is being sold at an advertised price which actually do not reflect now the real economic value of the ash, compared with other valuable broadleaved species, highly appreciated for veneer, which get higher prices.

Literature

Ajzen, I. (1991). "The theory of planned behavior." Organizational behavior and human decision processes 50(2): 179-211.

Chandelier, A., N. Delhaye and M. Helson (2011). "First report of the ash dieback pathogen Hymenoscyphus pseudoalbidus (Anamorph Chalara fraxinea) on Fraxinus excelsior in Belgium." Plant Disease 95(2): 220-220.

Kirisits, T., M. Matlakova, S. Mottinger-Kroupa, T. L. Cech and E. Halmschlager (2009). The current situation of ash dieback caused by Chalara fraxinea in Austria. Proceedings of the conference of IUFRO working party, Citeseer.

Lõhmus, A. and K. Runnel (2014). "Ash dieback can rapidly eradicate isolated epiphyte populations in production forests: a case study." Biological Conservation 169: 185-188.

Mitchell, R., J. Beaton, P. Bellamy, A. Broome, J. Chetcuti, S. Eaton, C. Ellis, A. Gimona, R. Harmer and A. Hester (2014). "Ash dieback in the UK: a review of the ecological and conservation implications and potential management options." Biological Conservation 175: 95-109.

Ogris, N., T. Hauptman, D. Jurc, V. Floreancig, F. Marsich and L. Montecchio (2010). "First report of Chalara fraxinea on common ash in Italy." Plant Disease 94(1): 133-133.

Todnem By, R. (2005). "Organisational change management: A critical review." Journal of Change Management 5(4): 369-380.

Jaques, Tony. "Reshaping Crisis Management: The Challenge for Organizational Design." *Organization Development Journal* 28.1 (2010): 9+. *Questia*. Web. 14 Feb. 2016.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 33 – 52.

Ash dieback in Austria – history, current situation and outlook

B. HEINZE[1,*], H. TIEFENBACHER[2], R. LITSCHAUER[1,†], T. KIRISITS[3]

[1] *Austrian Federal Research Centre for Forests (BFW), Department of Genetics, Vienna, Austria,* [2] *Grafenegg forest estate, Grafenegg, Austria,* [3] *Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences, Vienna (BOKU), Hasenauerstraße 38, A-1190 Vienna, Austria*
** corresponding author: berthold.heinze@bfw.gv.at*

Abstract

In this contribution, we aim to summarize the current knowledge on the main ash (*Fraxinus*) species in Austria and on the situation of ash dieback, caused by *Hymenoscyphus fraxineus*. *F. excelsior* is of considerable importance as a timber species. It occurs on a wide variety of sites, in different ecological contexts. Planting of ash used to be encouraged until about ten years ago, as it seemed very suitable to replace conifer stands in lowland areas. The species is severely affected by ash dieback, just as the more marginal species, *F. angustifolia*, while *F. ornus*, the third ash species native to Austria, is not endangered. The progress of the disease has been very fast, affecting practically the whole territory of Austria within very few years from approximately 2005 onwards. It now questions the continuity of timber production in forest enterprises where ash is the main species, impairs other ecosystem services and has numerous negative ecological consequences. Likewise, it has affected nursery plant production heavily. Landscape level effects of the impact of ash dieback are possibly reflected in declining pollen loads in the air. In the forest, variations in disease intensity are observed from year to year, and between moister and drier regions; yet, the disease seems to progress in most individual trees. Solitary trees in cities and in the cultural landscape are generally less affected by ash dieback. Clones of *F. excelsior* in three seed orchards have been assessed for crown damage levels over three years. These observations indicate that some clones are more resistant or tolerant to ash dieback than others. Consequently, a resistance breeding project for common ash has recently been initiated. There is little evidence for important genetic differentiation among population or seed stands within Austria. Options for adapting silvicultural strategies and for conserving *F. excelsior* are discussed.

Keywords: *Fraxinus excelsior*, *Fraxinus angustifolia*, *Fraxinus ornus*, *Hymenoscyphus fraxineus*, *Chalara fraxinea*, noble hardwoods, silviculture, disease management, resistance, genetic differentiation, conservation

Introduction

Austria is situated at the crossroads of major climate regions in Europe, with the Alps acting as a central barrier, as an eco-region of its own, but also as a fusion and transition zone. This is reflected in the flora and fauna, and in the diseases associated with plants and animals. In this contribution, we aim to summarize the present local situation (as of September 2016) of ash (*Fraxinus* spp.) dieback caused by the invasive alien ascomycete pathogen, *Hymenoscyphus fraxineus*. The ecological and socio-economic importance of ash species, particularly of *F. excelsior*, is described, followed by a description of the successive emergence, the hosts and the impact of the disease, as well as a brief review of studies on the aetiology of ash dieback in Austria. The baseline for intra-specific (genetic) effects on *F. excelsior* is given by recent molecular marker studies. Indications for possible genetic resistance in *F. excelsior* to *H. fraxineus* based on research studies and observations in forest practice are reviewed and weighted. Current management (silvicultural) options and conservation strategies for ash are presented and discussed, together with an outlook to the immediate future, regarding both the further development of the disease and ongoing research initiatives.

Importance of ash

Common or European ash (*Fraxinus excelsior*) is widely distributed in Austria, together with *F. angustifolia* (narrow-leaved ash; only along rivers in the eastern border regions) and *F. ornus* (flowering or manna ash; rare, mainly along the southern borders to Slovenia and Italy). *F. excelsior* occurs in many forest types, from the lowlands up into the mountains. It prefers nutrient-rich, deep soils with higher moisture levels, which typically

---

[†] Our colleague Rudolf Litschauer (retired from BFW) passed away in August 2016. This contribution is dedicated to his memory.

occur in floodplain forests, along streams, ravines and gorges, and on lower slopes. In the mountains, it is found at elevations up to approximately 1200 m (exceptionally 1600 m). However, the spectrum of site conditions suitable or tolerable to ash is much wider and includes also drier sites, and some of its preferences are only matched by early-successional tree species of low importance for timber production (e.g., by silver birch – *Betula pendula* – or aspen – *Populus tremula*).

According to the website www.flora-austria.at, up to 32 vegetation associations have a characteristic *F. excelsior* component. The most important ones are hardwood floodplain forests along big rivers (originally with elm, *Ulmus* spp., and oak, *Quercus robur*), ash-black alder (*Alnus glutinosa*) communities along smaller streams, and the ravine and gorge associations with typically *Ulmus glabra, Acer pseudoplatanus* and *Tilia platyphyllos*. In floodplain forests that are flooded for longer average times, in the warmer climates of the east, *F. excelsior* is replaced gradually by *F. angustifolia* (Zukrigl 1997). Typically, this is the case for the lower March, Leitha and Fischa rivers in the Pannonian region of Eastern Austria, and along the easternmost stretches of the Danube. Here, the Danube gradually loses its character of a mountain stream, flooding episodes of the Danube block the water of the tributaries (March and Leitha) for longer time periods, resulting in prolonged flooding of forests in spring and summer. The two ash species also hybridize where their distribution ranges overlap (Zukrigl 1997, Lexer et al. 2004). Ash-black alder forest strips along smaller rivers and streams are as extensive in surface area as are floodplain forests (50,000 hectares each, Gschwantner et al. 2010), and the ravine and gorge associations are found all over the country in hills and mountains. Apart from all these forest types, *F. excelsior* is also often interspersed in other, related forest communities. A specific type of forest are “hilltop ash forests” (in German, “Gipfeleschenwälder”; Willner 1996), where ash outcompetes European beach (*Fagus sylvatica*) and often forms pure stands. These often occupy atypical sites, considering what is written above: hilltops west of Vienna with shallow soils, and probably less advantageous water conditions. Willner (1996) could show, however, that moisture there is not only received regularly (and particularly, in spring) in the form of rain, but also often as fog, and that the plant associations generally resemble those of moister sites.

A forestry tradition is the distinction between “limestone” and “water” ash (in German “Kalkesche” and “Wasseresche”, respectively). The two main types of sites for *F. excelsior* differ remarkably in soil moisture. This distinction of typical sites along rivers, streams and in gorges (“water ash”) and sites with rendzina soil (“limestone ash”) has led to speculation that these have led to the evolution of ecotypes with different characteristics. Trees from drier (limestone) hillsides are also thought to yield timber of lower quality. Yet, Weiser (1995) could show with reciprocal transplants that there are no such ecotypes or “races”. Ash needs sufficient amounts of water especially in May and June, but it can tolerate lower amounts in other months. More water is needed in order to sustain vigorous growth because of high transpiration rates, but the species can survive and is competitive with less water as well, as under the conditions at drier limestone sites in Austria.

The third ash species native to Austria, *F. ornus*, reaches one edge of its natural distribution range in Southern Austria. It mainly occurs in Southern and Eastern Carinthia, on steep, rocky, warm and dry sites on limestone, where it is usually associated with European hop-hornbeam (*Ostrya carpinifolia*; Zukrigl 1997). *F. ornus* is not important for timber production, but sometimes planted as an ornamental tree. Among non-native ash species, green or red ash (*Fraxinus pennsylvanica*) was planted experimentally along the lower reaches of the Danube and March. This was discontinued because of lack of yield and quality timber, but the species became naturalized and is now viewed critically as an invasive alien tree species. White ash (*Fraxinus americana*) is rarely planted as shade tree or in arboreta. Manchurian ash (*Fraxinus mandshurica*) and Korean ash (*Fraxinus rhynchophylla*), the natural hosts of *H. fraxineus* in Asia, are not known to be cultivated, and there are no historical records either.

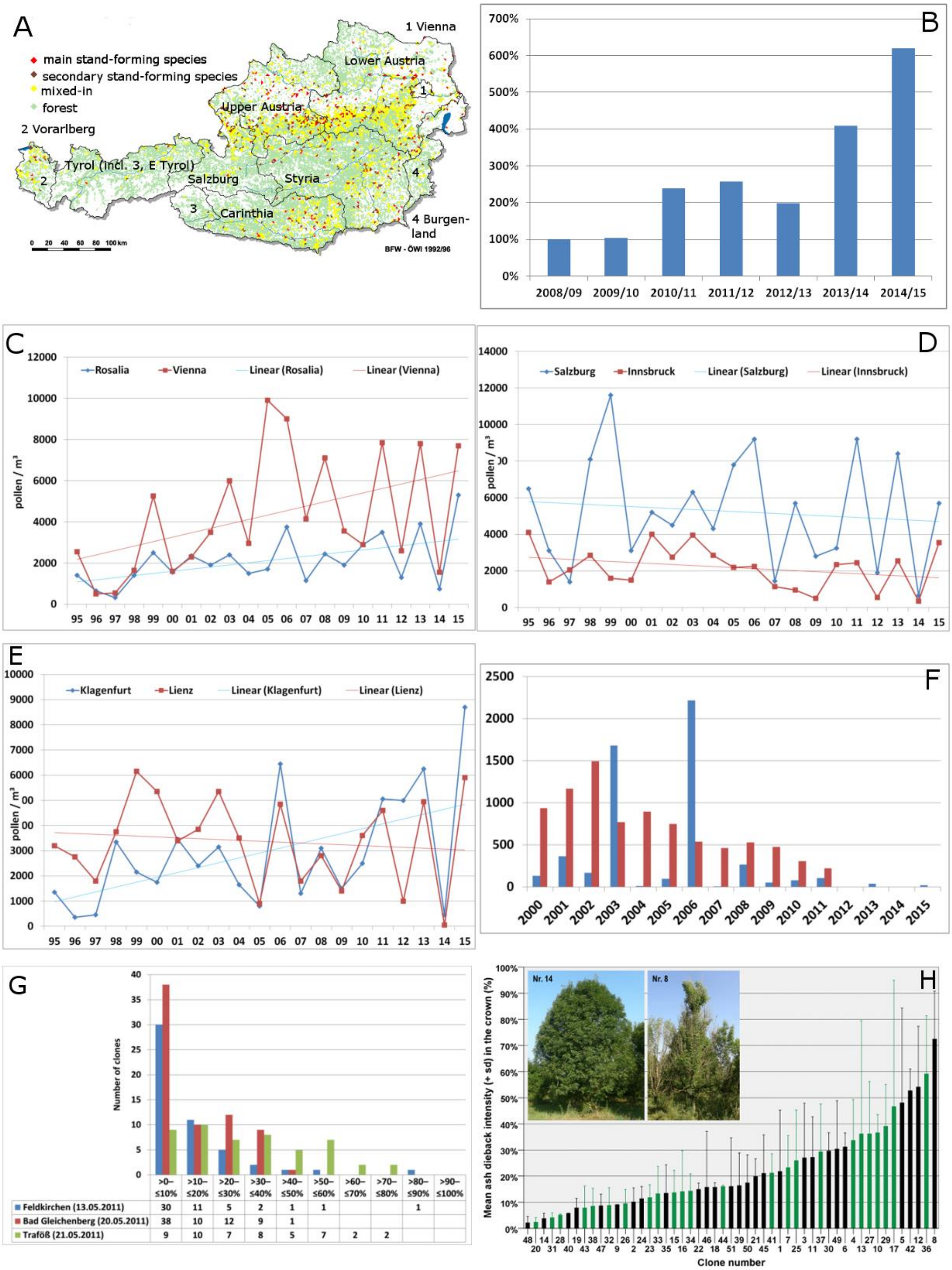
The importance of ash, mainly *F. excelsior*, in forests and for forestry in Austria is quite high. The Austrian Forest Inventory (www.waldinventur.at; Gschwantner et al. 2010) calculated 23,705,000 (±1,439,000) cubic metres of growing stock of wood in managed forests (covering a total of 3.367 million hectares) for ash in the period 2007-2009 (the latest available data). It thus has a share of 2.1 % among all tree species and holds the third position among hardwoods: European beech, *Fagus sylvatica*, with 9.6 %, and – narrowly so – the oak species, *Quercus* spp., with 2.4 %, have more growing stock; the maple species, *Acer* spp., with 1.3 % have considerably less). Ash trees thus represent about 10 % of the hardwood growing stock. Based on number of stems, it even comes second, with a share of 3.1 % among all tree species. In the latest inventory period reported (compared to the previous one, 2000-2002), ash was still increasing its growing stock (by a calculated 4,295,000 ± 542,000 m³) – a trend that was already seen in previous inventory periods. Total increment per year was estimated at 833,000 m³, and utilization at 309,000 m³. The latter figure represents already a steep increase compared to the previous periods (i.e., 184,000 m³ in 2000-2002, and 190,000 in 1992-1996), and this may already reflect the onset of the ash dieback epidemic (apart from market demand fluctuations and many other factors). The total number of ash stems was continuously increasing in the last three reporting periods (74,021,000 in 1992-1996; 89,145,000 in 2000-2002; and 105,969,000 in 2007-2009). In relation, this means that there has been a trend for an increase in growing stock per individual stem in the three periods (from 0.204 to 0.218 to 0.224 m³ per stem), though with a large error margin. This situation – more ash planted/regenerating (increasing stem number), together with increasing utilization, and increasing volume per individual stem – is difficult to interpret, though.

The share of ash is particularly high in the provinces of Upper Austria, Lower Austria, Vorarlberg and Vienna, and lowest in Tyrol (Figure 1A, data of the 1992-1996 inventory period). Regionally, the distribution data for ash in the Austrian Forest Inventory indicates a higher frequency of pure stands or stands with a significant share of ash in the flat areas surrounding the big rivers (Danube, Mur, Leitha and March) in the north, east and south of the country. An even larger overall number of stands with ash is found at lower elevations in the mountains, especially in the Northern and Southern Limestone Alps. In these areas, mixed stands are more typical. The inner Alps (large parts of Tyrol, Carinthia, Styria and Salzburg) rarely host stands with a high share of ash, but in the very west, in Vorarlberg near Lake Constance and along the Rhine and its tributaries, the species is again common. The ecological amplitude of the species is thus quite wide in Austria. The relative rareness of *F. excelsior* in inner Alpine valleys is most likely due to its sensitivity to late frosts (and its demand for higher temperatures in general).

Locally, the importance of ash for ecosystems and forest owners is immense. Several sizable forest holdings are situated in the floodplains along the Danube and Rhine, and their major tributaries. They depend on ash to a large degree. Apart from the "softwood" stands very close to the river (with willows – *Salix* spp., and poplars – *Populus* spp.), the more mature soils of the "hard" floodplain forests traditionally hosted oak, elm and ash as the main timber species. With the vanishing of elm (mainly field elm, *Ulmus minor*) due to Dutch elm disease caused by *Ophiostoma novo-ulmi* (Kirisits and Konrad 2004) and the dieback of ash, the owners of these forests find themselves in a very difficult economic situation, because of the lack of choice of suitable timber species. For instance, in the area of the Grafenegg forest estate (Lower Austria), a privately-owned enterprise of 4,466 ha forests, a growing stock of 200,000 m³ ash is endangered and to a large degree already affected. Similarly, in floodplain forests along the March (Lower Austria), for example in those managed by the Foundation Prince Liechtenstein in Hohenau an der March, *F. angustifolia*, which is also susceptible to ash dieback (Kirisits et al. 2010a), is the most important timber species (Damm 1997).

Ash is of some importance for many forest owners, for timber production, but also for other reasons (e.g., stabilization of riverbanks and slopes, source of fodder for wildlife, increasing habitat quality), even if it is usually not the major species in their forests. During the last decades, it was one of the most frequent tree species used in afforestations. This was in order to replace Norway spruce (*Picea abies*) in areas where this conifer is not native, following concepts of "close-to nature silviculture". Ash has been regenerated naturally in most other situations. Farmers often utilize the ash-black alder forests for valuable timber and for fuelwood. During the recent "boom years" of biomass heating systems and combined heat-power plants, many farmers have installed such systems, and may now, with ash dieback, face irregularities in fuelwood supplies.

Ash species are of considerable importance for nature conservation, as components of rare forest ecosystems and as hosts of or habitats for numerous organisms. For example, *F. excelsior* is the mandatory fodder plant of the red listed and threatened butterfly *Euphydryas maturna*, the scarce fritillary. Common ash is also an important element of the cultural landscape in many parts of Austria, including areas such as the inner Alps where it is rare in forests. It is planted in hedgerows or as a solitary tree, or e.g., near the farmhouse. Because of its nutrient-rich foliage, it provided fresh or dry fodder for domestic animals (lopping or pollarding). European ash, its varieties and occasionally other ash species are commonly used as ornamental trees in the urban green and for plantings along roads and in the landscape.



	>0–≤10%	>10–≤20%	>20–≤30%	>30–≤40%	>40–≤50%	>50–≤60%	>60–≤70%	>70–≤80%	>80–≤90%	>90–≤100%
Feldkirchen (13.05.2011)	30	11	5	2	1	1			1	
Bad Gleichenberg (20.05.2011)	38	10	12	9	1					
Traföß (21.05.2011)	9	10	7	8	5	7	2	2		

Figure 1A Distribution data of ash species (mainly *F. excelsior*) from the Austrian National Forest Inventory, 1992-1996 period. **Figure 1B** Relative increase of ash logging at Grafenegg forests from 2008/2009 to 2014/2015, compared to the original level (2008/2009 = 100 %). **Figure 1C-E** Pollen intensity (density as counted from pollen traps) in three regions of Austria, 1995-2015. C, Eastern Austria (stations Vienna and Rosalia hills); D, Stations in the Northern Alps (Salzburg and Innsbruck); E, Stations in the Southern Alps (Klagenfurt and Lienz). 'Linear': linear trend line. Data source: European Aeroallergen Network (EAN) database (https://ean.polleninfo.eu/Ean/en/start), K. Bastl, U Gartner, S. Bortenschlager, H. Zwander, compiled by R.

Litschauer. **Figure 1F** Seed harvest amounts and plant production of ash in Austria, 2000-2015. Red columns, thousands of plants produced in nurseries; blue columns, amounts of seed harvested in kg. Data source: T. Baschny, Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management, personal communication. **Figure 1G** Distribution of *F. excelsior* clones in each of the three Austrian seed orchards (in Feldkirchen, Bad Gleichenberg and Traföß) among 10 %-damage classes (mean crown dieback intensity according to assessments in May 2011). Bars represent the number of clones in each damage class per seed orchard. The figure was originally shown in Freinschlag (2013) and Freinschlag and Kirisits (2014). **Figure 1H** Mean ash dieback intensity (+ standard deviation) in the crown (as percentage) by *F. excelsior* clone on 3-4 June 2015 in the seed orchard in Feldkirchen an der Donau (Upper Austria, 2-4 ramets per clone, 184 trees in total). The photos show a ramet of one of the least damaged clones (no. 14) and one of the most severely damaged clone (no. 8). Green bars mark clones which produced seed in 2015 and were harvested to initiate a progeny trial as part of the project “Esche in Not” (“ash in distress”, see http://www.esche-in-not.at/). The figure was adapted from Kirisits et al. (2016).

Successive emergence, aetiology and hosts of ash dieback

The first unambiguous observations of symptoms of ash dieback caused by *H. fraxineus* (shoot dieback, necrotic lesions in the bark, wood discoloration) were made in 2005, at a few sites in the provinces of Lower Austria, Upper Austria and Salzburg, on young *F. excelsior* trees in afforestations (Cech 2006a, b). Also in 2005, massive premature leaf shedding of ash, occurring already in late August and early September, was observed in parts of the provinces of Lower and Upper Austria; the same happened 2006 in parts of Styria (Cech 2005, Kirisits et al. 2009). Originally, this phenomenon was thought to be caused by powdery mildews (Cech 2005), but as *H. fraxineus* also causes leaf symptoms (Kräutler and Kirisits 2012, Schwanda and Kirisits 2016), this was likely the first widespread indication of ash dieback (Kirisits et al. 2009, 2010b, 2011). In subsequent years, obvious early leaf shedding occurred regularly in various parts of the country, particularly in the Alps (Kirisits et al. 2010b, Kirisits and Freinschlag 2012). Intensive dieback of shoots, twigs and branches of ash trees of all ages occurred for the first time in 2006 over wide areas in the provinces of Lower and Upper Austria (Cech 2006b), and in 2007 in Styria (Kirisits et al. 2009). In subsequent years the disease occurred steadily at new places and gradually increased in incidence and severity (Kirisits 2008, 2011, Kirisits et al. 2008, 2011, Cech et al. 2012).
Although the spread of the ash dieback epidemic was not followed in every detail, observations and investigations suggest that the disease emerged later in western, southern and south-eastern parts of the country, compared to Eastern and Northern Austria (Table 1; Kirisits 2011, Kirisits et al. 2011). By 2008 symptoms were observed in all Austrian provinces (Table 1; see Figure 1A for the location of the provinces), and at least by 2010 the disease was likely present in all political districts of the country. A survey in July 2010 in parts of Carinthia and Eastern Tyrol indicated that some localized areas in these provinces were still free of ash dieback (Kirisits 2011, Kirisits et al. 2011). A re-visit of some of these areas in Eastern Tyrol in summer 2014 confirmed the presence of *H. fraxineus* there, up to the upper elevation range of common ash at 1600 m asl. in the Virgental (T. Kirisits, unpublished observations). The pathogen is now distributed in the entire ranges of *F. excelsior* and *F. angustifolia* (on which it was first recorded in 2008; Kirisits et al. 2010a) in Austria.

The observations in Austria fit with the data from surrounding countries (Kirisits at al. 2016), where written and informal reports suggest the presence of the disease in the Czech Republic in 2002, in Slovakia in 2004, in Slovenia and Southwestern Germany in 2006, in Hungary and Switzerland in 2007, in Bavaria (Southeastern Germany) in 2008, and in Croatia and Northern Italy in 2009. Symptoms of ash dieback were, however, first observed as early as in 1992 in Northeastern Poland and in 1995 in Lithuania, thus suggesting the possibility of an undiscovered lag phase, at least in some countries, before symptoms became widely apparent. This may have happened within Austria as well.

As in other countries, the aetiology of ash dieback was initially poorly understood and its causes were debated (Cech 2006b, Cech and Hoyer-Tomiczek 2007), but soon, *H. fraxineus* was confirmed as causal agent (Halmschlager and Kirisits 2008, Kirisits et al. 2008, 2009, Matlakova 2009). *H. fraxineus* (at that time referred to as *Chalara fraxinea*) was for the first time isolated in Austria in June 2007, at one locality in Upper Austria and one in Styria (Table 1; Halmschlager and Kirisits 2008, Kirisits et al. 2009). Subsequent surveys showed that the pathogen was already widespread in the country (Halmschlager and Kirisits 2008, Kirisits et al. 2008, 2009, 2011). From June 2007 until June 2011, it was detected in all Austrian provinces, at 144 localities in total (Table 1), including also forest nurseries (Kirisits et al. 2012). Initially, in 2007, *H. fraxineus* was rarely recovered (due to using quite old material with late symptoms for fungal isolation), but from 2008 onwards, when isolations

Table 1 First records of symptoms of ash dieback and of *Hymenoscyphus fraxineus* isolated from symptomatic ash trees trees[1] in each of the nine Austrian provinces[2] (see Figure 1A for their location).

Austrian province	First record of ash dieback symptoms	First record of *H. fraxineus*		Recorded *Fraxinus* hosts[3]	Number of localities[6]
		Month & year	Locality, political district		
Burgenland	2008[4]	May 2010	Breitenbrunn, Eisenstadt-Umgebung	*F. excelsior*, *F. angustifolia*, *F. ornus*, *F. pennsylvanica*, *F. americana*, *F.* cf. *latifolia*, *F.* cf. *velutina*	4
Carinthia	2008[4]	August 2008	Saberda, Klagenfurt-Land	*F. excelsior*	16
Lower Austria	2005[4]	August 2007	Langau, Scheibbs	*F. excelsior*, *F. angustifolia*, *F. pennsylvanica*	42
Salzburg	2005[4]	October 2008	Fuschl, Salzburg-Umgebung	*F. excelsior*, *F. excelsior* 'Pendula'	15
Styria	2006[4]	June 2007	Altaussee, Liezen	*F. excelsior*, *F. excelsior* 'Pendula'	16
Northern Tyrol	2008[5]	October 2009	Brixlegg, Kufstein	*F. excelsior*	11
Eastern Tyrol	2010[4]	July 2010	Dölsach, Lienz	*F. excelsior*	2
Upper Austria	2005[4]	June 2007	Edt bei Lambach, Wels-Land	*F. excelsior*	13
Vienna	2007[4]	January 2008	Neuwaldegg, Hernals	*F. excelsior*, *F. excelsior* 'Pendula', *F. angustifolia*, *F. pennsylvanica*, *F. americana*, *F.* cf. *latifolia*, *F.* cf. *velutina*	17
Vorarlberg	2008[5]	May 2009	Götzis, Feldkirch	*F. excelsior*	8
Total		-	-	-	144

[1] Isolations were mainly made from woody parts (at two localities in Lower Austria only from leaf rachises).

[2] For the province of Tyrol, data are shown separately for Eastern and Northern Tyrol.

[3] On *F. ornus*, *H. fraxineus* was only isolated from leaves at a site in Burgenland, and isolations from woody parts of *F. americana*, *F.* cf. *latifolia* and *F.* cf. *velutina* were made from seedlings during nursery production (Vienna) and from seedlings planted at a site in Burgenland.

[4] Unambiguous observations by T.L. Cech (BFW) or T. Kirisits (IFFF-BOKU). The records do not necessarily reflect an exact time sequence of the occurrence of ash dieback in the various provinces, as they were made during opportunistic and not systematic surveys. It is, however, safe to conclude that ash dieback occurred later in Western, Southern and South-Western Austria (provinces of Carinthia Tyrol and Vorarlberg, political district Murau in Styria, political district Lungau in Salzburg) than in other parts of the country.

[5] Personal communications by staff of the Provincial Forest Authorities of Tyrol (R. Seitz and C. Schwaninger) and Vorarlberg (A. Kapp).

[6] Number of localities where *H. fraxineus* was isolated from diseased ash trees from June 2007 to June 2011. The differences in the number of records of the ash dieback pathogen in the various provinces do not allow inferring about the intensity of ash dieback in various parts of Austria but just reflect differences in the intensity of sampling. The pathogen has since about 2012 been occurring practically everywhere in Austria.

were made from shoots, twigs and stems showing early symptoms of disease, it was the most consistently and most frequently isolated fungus (Kirisits et al. 2009, 2012).

The pathogenicity of *H. fraxineus* to *F. excelsior* was confirmed in wound inoculation experiments, thus fulfilling Koch's postulates for *H. fraxineus* as causal agent of ash dieback (Kirisits et al. 2008, 2009, Matlakova 2009). In one inoculation experiment the fungus even proved to be able to infect unwounded current-year shoots and artificially exposed leaf scars (Kräutler et al. 2015). The association of *H. fraxineus* with symptoms on woody parts of *F. angustifolia*, *F. pennsylvanica* and *F. americana*, as well as with leaf symptoms on *F. excelsior*, *F. angustifolia*, *F. pennsylvanica* and *F. ornus* was also proven by fungal isolations from naturally infected plants and wound inoculation experiments (Kirisits et al. 2009, Matlakova 2009, Kräutler and Kirisits 2012, Piribauer 2013, Treitler 2014, Kirisits and Schwanda 2015, Kirisits 2016, Kirisits and Cech 2016, Schwanda and Kirisits 2016, T. Kirisits and K. Schwanda, unpublished data). Likewise, the fungus was occasionally isolated from seedlings of *Fraxinus* cf. *latifolia* (Oregon ash) and *Fraxinus* cf. *velutina* (velvet ash) showing shoot dieback and necrotic lesions in the bark (Kirisits and Cech 2016, T. Kirisits, unpublished data), but the identity of the ash species requires confirmation. Moreover, in September 2016 leaf symptoms on two-year-old *F. mandshurica* seedlings resembling those described by Drenkhan et al. (2016) in the species' natural range were observed, but it is as yet not clear whether this damage is linked to *H. fraxineus* (T. Kirisits, unpublished observations). Following wound inoculation, *H. fraxineus* did not cause necrotic lesions on shoots of *Forsythia* x *intermedia* (forsythia), *Ligustrum vulgare* (privet) and *Syringa vulgaris* (lilac) (Kirisits and Cech 2016, R. Bzdyk and T. Kirisits, unpublished data), indicating that its host range may be restricted to species in the genus *Fraxinus*. These results are in agreement with inoculation experiments on oleaceous species in other countries (Gross et al. 2014; Madigan et al. 2015).

Records of disease incidence and severity due to natural infections and inoculation experiments conducted in Austria suggest that *F. excelsior* and *F. angustifolia* are highly and nearly equally susceptible to *H. fraxineus* or that *F. angustifolia* is perhaps slightly less susceptible (Kirisits et al. 2009, 2010a, Matlakova 2009, Kräutler and Kirisits 2012, Piribauer 2013, Treitler 2014, Schwanda and Kirisits 2016). Afforestations of *F. angustifolia* in floodplain areas along the March have been severely affected since at least 2007 (Kirisits et al. 2010a, Piribauer 2013); however, damage levels have remained relatively low on mature trees (Cech et al. 2012 [site Hohenau 2], H.J. Damm, Foundation Prince Liechtenstein, personal communication). Although *F. ornus* was to some degree susceptible to *H. fraxineus* in stem and leaf inoculation experiments (Kirisits et al. 2009, Matlakova 2009, Schwanda and Kirisits 2016), symptoms due to natural infections have only been observed occasionally on leaves and never on woody parts; therefore, this ash species is most likely not threatened by the disease (Kirisits and Schwanda 2015, Kirisits 2016). On *F. pennsylvanica*, ash dieback was confirmed only occasionally on seedlings and young trees, and this species appears to be much more resistant than *F. excelsior* and *F. angustifolia*; however, inoculation experiments have clearly confirmed its susceptibility to *H. fraxineus* (Piribauer 2013, Treitler 2014). The susceptibility of the remaining ash taxa (*F. americana*, *F.* cf. *latifolia* and *F.* cf. *velutina*) to ash dieback in Austria cannot be assessed precisely at the moment, because *H. fraxineus* was isolated only occasionally from very young seedlings.

Research regarding the progression and consequences of ash dieback

Development and impact of ash dieback – Disease monitoring data and observational evidence

The temporal development and impact of ash dieback over time is mainly documented by observational evidence, experience and reports by practitioners, and to some degree by monitoring data. In 2007, shortly after the onset of ash dieback, a survey on 50 plots in mature ash stands in Lower Austria confirmed the wide extension of the disease; mean crown dieback intensity was below 25 % in most stands, tree mortality was recorded in eight stands, and there were still three stands without any dieback symptoms (Cech 2008, Cech et al. 2012). In 2008, on 14 of the 50 plots, representing the major ecoregions of Lower Austria, 20 trees each were permanently marked, and the development of crown dieback has been monitored since then, initially annually but later at irregular intervals (Cech et al. 2012, Keßler et al. 2012, T.L. Cech, BFW, personal communication). A slow progress of the disease was documented from 2008 (mean crown dieback intensity: 11 %, range per plot: 1-34 %) to 2010 (mean crown dieback intensity: 16 %, range per plot: 2-38 %) on these permanent monitoring plots; on three of the 14 plots one tree had died (Kirisits et al. 2011, Keßler et al. 2012). Stands in the eastern, dry regions of Lower Austria were less severely affected than stands in the moister western parts of the province; this trend was already observed in the survey in 2007 (Cech 2008, Cech et al. 2012, Kirisits et al. 2011, Keßler et al. 2012). Sporulation of and infections by *H. fraxineus* are generally facilitated by moist soils and high air humidity, which likely explains this pattern (Kirisits et al.

2011, Keßler et al. 2012). Monitoring results from these plots in subsequent years have not yet been published.

Interestingly, during these years of emergence, expansion and intensification of ash dieback, there were many summer months with high precipitation, which may have accelerated disease development (see also Cech 2005). As an example, in St. Pölten in central Lower Austria, among the six months from April to September, there were four months with more than average precipitation in 2005, four also in 2006, three in 2007, four in 2008, three in 2009, five in 2010 (a very wet year, in which premature leaf shedding was very intensive; Kirisits et al. 2010b), only one in 2011, four in 2012 and in 2013, five in 2014, and only one in 2015 (a record hot year, in which premature leaf shedding was inconspicuous); the values were on some occasions several-fold above the 1981-2000 averages (data from www.zamg.ac.at).

As on the monitoring plots in Lower Austria, slow development of ash dieback was observed in three clonal seed orchards of *F. excelsior* from 2009 to 2011 (Kirisits and Freinschlag 2012, Freinschlag 2013, Freinschlag and Kirisits 2014; see also section "*Genetic resistance to ash dieback*" below). These surveys indicated partial recovery of many ash trees due to the formation of epicormic shoots in the crown in response to infection. However, a more recent damage assessment in the orchard in Feldkirchen an der Donau (Upper Austria) documented a doubling of mean crown dieback intensity from 11.3 % in July 2011 (Freinschlag 2013, Freinschlag and Kirisits 2014) to 22.5 % and for the first time mortality of ramets (three of 187) in June 2015 (T. Kirisits, unpublished data).

The development of ash dieback in Austria during the last five years is not well documented by monitoring data. Generally, a gradual rise in disease intensity was observed in forests; in some regions and years, disease progression was accelerated. Apart from a possible correlation with wetter summers, this is likely due to the increasing prevalence and severity of necrotic lesions and wood discoloration at the stem base and root collar. These symptoms were initially linked to species of *Armillaria* (honey fungus), but are now considered to be also primarily caused by *H. fraxineus* (Husson et al. 2012, Kirisits 2013a, Chandelier et al. 2016). *Armillaria* species usually also occur in connection with these collar symptoms, cause butt and root rot, destabilize trees rapidly and make them prone to fall over (Husson et al. 2012, Chandelier et al. 2016, Kirisits et al. 2016, Lenz et al. 2016). Although no research on the aetiology of root collar symptoms has been conducted in Austria and their occurrence has not been systematically surveyed, observations and reports by practitioners show that they are widespread. As in other countries (Chandelier et al. 2016), collar infections, and not crown damage, are now thought to be the main drivers of decline and mortality of ash trees. In contrast, ash bark beetles (*Hylesinus varius*, *H. crenatus*, *H. taranio* and *H. wachtli orni*; valid species names according to M. Knížek, personal communication) appear to be only secondary damaging factors (as shown by Lenz et al. [2016] in Germany), colonizing mainly recently died trees, though Pfister (2012) claims that they can also infest living trees for breeding.

Ash dieback generally impacts younger trees more severely and more rapidly than older ones (McKinney et al. 2014, Lenz et al. 2016). Particularly, natural regeneration, seedlings and saplings are severely damaged within short time. For example, in a progeny trial that had been established in March 2014 with 400 two-year-old *F. excelsior* seedlings at a forest site in Upper Austria (Stadl-Paura), 81 % of the plants showed symptoms and 13 % were dead in August 2015 (T. Kirisits, unpublished data). Mortality is also common in trees up to 40 years, but even old ash trees are observed to die off at increasing rates (Kirisits et al. 2016). Even if infected ash trees survive, their growth and timber quality can be severely impaired, due to forking and wood discoloration (originating from infections of epicormic shoots from which *H. fraxineus* grows into the stem), and collar infections decrease the quality of the lowermost part of the butt log (Kirisits 2013a). All in all, ash dieback has become the most important forest disease of hardwood trees in Austria in recent years, and it seriously questions the future use of *F. excelsior* as an economically and ecologically valuable noble hardwood species.

In the monitoring plots in Lower Austria, it was found that ash dieback intensity is influenced by moisture (Cech 2008, Kirisits et al. 2011, Cech et al. 2012, Keßler et al. 2012). Equally, it is observed that the disease is usually more intense on sites with moist soil ("water ash") and climate than on sites with dry soil ("limestone ash") and climate (Kirisits et al. 2016). As mentioned, high moisture levels are likely favouring sporulation of *H. fraxineus* and conditions for infections. Particularly, root collar lesions (which accelerate mortality) occur much more frequently on moist or wet sites than on dry sites (Kirisits et al. 2016).

As in other countries (Thomsen 2014), ash dieback is a less severe problem on trees in the open landscape and in the urban green (Kirisits and Cech 2016, Kirisits et al. 2016), specifically on large specimens, which are sometimes not at all, or only negligibly, affected by the disease. Some of such trees showing higher levels of dieback frequently regenerate their crowns by developing epicormic shoots, and they can survive for longer time. In these conditions, the pathogen infection pressure is likely reduced, due to mowing, mulching, plowing, or the removal of autumn leaves in cities, or due to wind exposure of the trees (Thomsen 2014, Kirisits and Cech 2016). Collar infections of *H. fraxineus* occur more rarely, and the honey fungus, important as a secondary damaging agent, is less common than in forests (Thomsen 2014, Kirisits and Cech 2016). This “disease escape” may provide some perspectives for long-term survival and thus conservation of ash.

Data from the Grafenegg forest estate

The onset and intensification ash dieback is also documented by local forest data and observations, for example from the forests of Grafenegg. These forests had already suffered heavily at the time of Dutch elm disease, when particularly *U. minor* was decimated (Kirisits and Konrad 2004). Elm (currently, there is more *U. laevis* than *U. minor*; opposite to the situation prior to the disease), has nowadays decreased to less than one percent, compared to the situation 50 years ago when it was more numerous than ash. Ash has taken its place. Due to ash dieback, a clear decrease in increment (volume gain) was observed in Grafenegg over the last approximately 10 years. Ash logging is currently done there predominantly in response to the disease, and it has increased six-fold within a few years (from 2008/2009 to 2014/2015; Figure 1B). An inventory of defoliation on 6,000 trees in Grafenegg in August 2014 has revealed that most trees (about 60 %) show some, but less than 20 % defoliation. Less than ten percent of the trees seem to be unaffected. Dead trees can be found in every stand. Defoliation is more severe in younger stands, where also mortality hits hardest. This, the lack of natural regeneration and the lack of new plantings of ash have already led to a shift in age class distribution.

The loss of ash as an economically viable tree species would mean a severe problem for Grafenegg forest estate and forest owners in similar situations. Ash timber prices have already declined by approximately 50 % in the last 15 years.

Analysis of pollen intensity data

The European Aeroallergen Network database (https://ean.polleninfo.eu/Ean/en/start) collects pollen intensity data for a number of tree species. An attempt is made here to compare the pollen intensities over periods of years before and after the emergence of ash dieback. In theory, if many shoots of an adult (flowering) tree are affected by the disease, there should be fewer flowers overall and less pollen released into the air. Flowers are born on the shoots of the previous year. *F. excelsior* trees tend to have functionally incomplete flowers; individual trees either produce male or female types preferentially, but hermaphroditic flowers exist as well. Data for three regions of Austria are presented in Figure 1C-E. In Eastern Austria (Figure 1C), stronger flowering was observed approximately every second or third year. Both stations show such variations in the same phasing. An overall increasing trend is visible. Yet, this trend is much stronger until 2005. Interestingly, the first year of some confirmed disease cases in Eastern Austria (2005) was followed by a year of high pollen intensity, which is absent elsewhere in this 20-year period. Since 2005, pollen intensities in “mast years” have remained at a lower, but still high, level.

A different pattern is observed just north of the main chain of the Alps, in Salzburg and Innsbruck (Figure 1D), which receive more Atlantic precipitation, and where average temperatures are much lower than in the east. In Innsbruck, only four intensive pollen years stand out (1999, 2006, 2011 and 2013), and due to the exceptionally strong year 1999, the general trend is a declining one. In Salzburg, differences in pollen production are evident and in phase with Innsbruck, but many of the peaks in pollen quantities are not as strong or not present at all.

South of the main chain of the Alps in Austria, in Klagenfurt (Carinthia) and Lienz (Eastern Tyrol), again a different pattern emerges (Figure 1E). Pollen production has been peaking since 2011 (in four out of five years) at both locations. While for Klagenfurt, the general trend is slightly declining, Lienz sees an overall increase. There is an overall tendency of lower pollen quantities in Klagenfurt than in Lienz from 1995 to 2005, while in the second decade (2005-2015) amounts of pollen were about equal or higher in Klagenfurt. Pollen intensities are not always in phase for the two cities.

Many factors may have contributed to the patterns of pollen intensities. Apart from weather conditions (temperature and precipitation), changes in the number of flowering trees present (planting and regeneration, as well as maturing of young trees, *versus* removal and timber harvest) will have the highest influence. Local circumstances (trees in the vicinity) will be most important in this regard. On top of that, overall tree health conditions may also contribute to flowering patterns. In the areas of Eastern and Northern Austria, the trend towards planting more ash and towards more natural regeneration, visible from inventory data (see section “Importance of ash” above) may have led to more and more young ash trees reaching flowering age. The discontinuation of the steady upwards trend after 2005 may indicate the gradual emergence and intensification of ash dieback during this time span in these regions. The increasing intensity of ash dieback in Western Austria may be reflected already in the pollen data from Innsbruck (where ash dieback was confirmed in 2010) and Salzburg (where the disease may have already been present in 2005, but the first unambiguous record was made in 2009). Pollen intensities are declining there; especially the minima are lower in the second 10-year period (2005-2015) than in the first one (1995-2015). The two examples from south of the main chain of the Alps (Klagenfurt and Lienz, where ash dieback was confirmed in 2008 and in 2010, respectively) are more difficult to interpret. This may have to do with the overall rarity of ash in these areas (although overall pollen intensities are not much lower). This rarity may lead to more chance effects. Local effects in Klagenfurt (more trees reaching maturity in the local surroundings?) may be an explanation for the pollen intensity patterns there. The three regions also differ in the onset of flowering in each year; the necessary temperature sums are reached between the 50th and 100th day of the year in the east, between the 65th and 110th day in the north, and only between the (65th) 75th and 110th day in the more alpine climates in the south (data not shown).

Seed harvest and plant production data

The impact of ash dieback is visible from diminishing numbers of ash seed harvested and plants produced in nurseries (data provided by T. Baschny, Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management, personal communication; see also Baschny and Strohschneider 2012). In 2006, the highest number of (commercial) seed harvests was reached in Austria (19 lots); before 2006, “good” ash seed years resulted in 11-16 harvests (the average 1997-2006 was 7.9). Since 2007, however, the number of seed harvests has dropped to one or two per year, with only one exception (2008: six harvests). The amount of seed harvested is given in Figure 1F. It is also evident from this data that there is less and less diversity in seed stands used. Whereas there used to be “preferred” stands for certain provenance regions (where harvests were done multiple times) even before 2006, only very few stands have been harvested, but repeatedly, since that year.

The initially high demand for ash seed, as visible at the start of the millennium in the data presented, was connected to the increasing demand for plants. This increase in demand for noble hardwoods (ash, sycamore maple – *Acer pseudoplatanus*, wild cherry – *Prunus avium* and other species) was a trend that started around 1990. Before that time, planting of conifers (mainly Norway spruce) in lowland areas was a common practice in Austria. Heavy windthrows (e.g., due to storms “Vivian” and “Wiebke” in 1990) and bark beetle catastrophes that followed (H. Krehan, BFW, personal communication; see also http://bfw.ac.at/rz/bfwcms.web?dok=9605) led to a gradual change in attitudes, but more importantly, to changes in subsidy schemes. Coinciding with the dawn of the climate change awareness, planting hardwoods was rewarded with subsidies in Austria from the 1990s onwards, leading to this rise in demand for plants. We are trying here to demonstrate the “rise and fall” of ash planting before and after the emergence of ash dieback with nursery plant production data. The “rise” is visible until 2002 (Figure 1F), when a peak was reached at approximately 1.5 million plants produced. Expectations of the nurseries for sales of ash plants were still high at that time, as there was a record harvest of seed in 2006. At roughly the same time, however, severe ash dieback set in, and thus plant production has declined to about 220,000 in 2011/2012 (the last available data). This is likely due to increased awareness of the disease, coupled with recommendations not to plant ash any longer. The trends are very much reminiscent of Dutch elm disease on *Ulmus* species in Europe in the 1970s.

The analysis of plant production data is complicated by movement of seeds and plants among EU member states, which includes, for example, movement of Austrian seeds for plant production into other countries and re-transfer of plants, and *vice versa*. For *F. excelsior*, the by far most important flow of trade into Austria concerns Northern Germany (forest reproductive material only): from 2003 to 2010, 854,515 plants of German provenance and 237,100 grown from Austrian seed sources were brought from Schleswig-Holstein into Austria (I. Strohschneider, BFW, personal communication). Given that ash dieback occurred slightly earlier in Northern Germany and Denmark than in Austria (Timmermann et al. 2011), a transfer of latently infected plants (which is likely) could have played a role for the geographical pattern and timeline of emergence of the disease in Austria (the first records were indeed made on planted seedlings; Cech 2006a,b). In the same time period, movements of ash plants (but much lower quantities) are also documented from Belgium, the Netherlands and Hungary into Austria (I. Strohschneider, BFW, personal communication).

Genetics of ash: populations and possible resistance to *H. fraxineus*

Population structure and genetic diversity of ash in Austria

First attempts at characterizing *F. excelsior* for its genetic diversity were made during the European Union-funded research project, "RAP – Realising Ash's Potential", 2001-2005 (Heinze 2001). Research conducted included analysis of chloroplast DNA for overall differentiation in Europe, a paternity study in a small isolated stand in the vicinity of Vienna, an investigation into genetic diversity among and within seed lots, and an analysis of clones selected for two seed orchards.

Results of the chloroplast DNA study suggest that eastern parts of Austria, where ash used to be a valued timber-producing species, share a common haplotype, H02, while there may be some variation in the Alpine region of the country, with two more, closely related haplotypes occurring in parts of the Eastern (H05) and Central/Western Alps (H03; Heuertz et al. 2004, 2006, Heinze 2007). The common haplotype H02 is distributed from Croatia to the Netherlands, while the others may be more restricted to Italy, Switzerland and France (H03), and to Northern Italy, Central Austria and Southeastern Bavaria (H05), respectively. The latter, more restricted haplotype H05 is common in *F. angustifolia* in Southern Italy, though, while for the two other haplotypes, there is complete sharing and a common geographical distribution among *F. excelsior* and *F. angustifolia*.

A small stand of rather young *F. excelsior* trees was investigated with a view to studying eventual pollen inflow. The approximately 20 trees of the stand were separated by 200 m from the next adult ash trees. This is a distance previously considered as an effective maximum for seed dispersal, and beyond the usual pollen dispersal distance for the genus *Fraxinus* (Richards 1997). Paternity analysis with microsatellite markers in seed collected from a single seed-producing female tree indicated that all pollen contributions to the seed came from trees outside of the stand, despite the rather high local density of male-flowering trees (Heinze et al. 2005).

The investigation of six seed stands is presented in detail elsewhere (Heinze and Fussi 2016). Briefly, samples of six commercial lots of 95-96 seeds each were obtained across Eastern Austria. According to the legal regulations, at least ten different trees have to be used in such commercial seed harvests in Austria. The seeds were kept separated according to their mother trees and sent to the BFW laboratory. Microsatellite analyses was done in order to check the family structures (whether all seeds allegedly originating from a single mother tree really showed compatible genetic patterns, and whether the 10-13 trees in the individual lots were likely to be genetically different from each other). Results indicated that not in all cases, the seeds in a single tree lot shared compatible marker patterns, and that not in all cases, the number of different trees allegedly used for seed harvest was correct (Heinze and Fussi 2016).

The analysis of the clones in the two seed orchards, in Feldkirchen (Upper Austria) and Bad Gleichenberg (Styria) with the same microsatellite markers indicated high overall genetic diversities in both orchards (Fussi et al. 2008). The allelic profiles of the markers in both seed orchards were very similar to local seed lots, thus suggesting that the clones in both orchards are highly representative for the regions where they were collected. Furthermore, the data also suggested that seed orchards can provide seed with enhanced genetic diversity, at least if all clones contribute genetically to the seeds (Fussi et al. 2008).

Use of local material for afforestations

To the knowledge of the authors, no provenance trials have ever been planted for ash in Austria. Seed is / was harvested separately for regions of provenance and altitude zones in Austria, and theoretically, plants should be used within the matching regions of provenance. However, in practice, the high number of regions of provenance and altitude zones deters nurseries from growing all seed sources. Importation of German provenances in high quantities (see section "*Seed harvest and plant production data*" above) hints at a similar issue. Certain stands in Austria are harvested preferentially, and plants produced with that seed are also used for neighboring regions of provenance. This leads to an unwanted effect, namely a decrease in overall genetic diversity. Of course, this does not apply to naturally regenerated ash. Unfortunately, the exact numbers of re- or afforestations with ash *versus* natural regeneration are not available. It may be concluded from the increase of ash in volume and stem number (see section "Importance of ash" above) until at least the forest inventory period 2007-2009 that there must have been considerable planting of ash, which is in line with the plant production data presented above (Figure 1F; see section "*Seed harvest and plant production data*").

Genetic resistance to ash dieback

Evidence for variation in genetic resistance or tolerance within *F. excelsior* to *H. fraxineus* in Austria mainly comes from investigations in three clonal seed orchards, in Feldkirchen an der Donau (Upper Austria), Bad Gleichberg and Trafößß (both in Styria). Damage assessments of crown dieback and premature leaf shedding were done from 2009 to 2011 on a total of 622 ramets belonging to 171 ash clones (Kirisits and Freinschlag 2012, Freinschlag 2013, Freinschlag and Kirisits 2014). Two of the three orchards (in Feldkirchen and in Bad Gleichberg) were included in the genetic diversity studies mentioned above. Considering only surviving trees, mean crown dieback in each of the orchards did not increase drastically during the observation period. In May 2011 mean dieback intensity was 14.2 % in Feldkirchen (n = 187 ramets), 13.5 % in Bad Gleichenberg (n = 234 ramets) and 31 % in Traföß (n = 201 ramets). In each plantation, disease intensity among clones ranged from almost no (< 5 %) to severe dieback. No single clone was totally unaffected though. In the assessments in May 2011 a large portion of clones in Feldkirchen (59 %) and Bad Gleichenberg (54 %) had a mean crown dieback intensity below 10 %, but clones with such low damage levels occurred less frequently in the youngest plantation in Traföß (18 %; Figure 1G). As it was observed for crown dieback, the intensity of premature leaf shedding varied greatly among clones in each year this trait was assessed (Kirisits and Freinschlag 2012, Freinschlag 2013, Freinschlag and Kirisits 2014). Rankings of clones according to the intensity of leaf shedding in September of different years were strongly and significantly correlated with each other. In none of the three orchards, root collar lesions were recorded.

The assessments indicated that *F. excelsior* genotypes vary greatly in resistance to *H. fraxineus*, which is in agreement with studies in other European countries (e.g., McKinney et al. 2014). However, it was also noted that overall damage levels in the seed orchards were lower than on trees of similar age in forests, for sure because of the lower infection pressure of *H. fraxineus* (see also section “*Development and impact of ash dieback – Disease monitoring data and observational evidence*” above). Freinschlag (2013) therefore recommended following the development of the disease and the change in the performance of the clones in the seed orchards in subsequent years.

In Feldkirchen, another crown dieback assessment was conducted in June 2015, which indeed showed an increase in disease intensity overall (mean: 22.5 %) and also for most of the single clones (Figure 1H). For the first time, mortality was observed (three of 187 ramets); however, none of the surveyed trees showed root collar lesions. Only 13 of 51 clones (25 %) had now a mean crown dieback intensity below 10 % (Figure 1H). A few clones were still negligibly damaged, and the five least damaged clones in 2012 (nos. 48, 20, 14, 31 and 28; Kirisits and Freinschlag 2015) were also the five least damaged ones in 2015 (Figure 1H), though their order changed slightly. The increase in crown dieback levels from 2011/12 to 2015, overall and on individual clones, can partly be explained by the increase of the size of the trees. Some of the dieback of woody parts, particularly in the lower crown, may thus be due to self-shading (which cannot be discriminated in the assessments from the effects of ash dieback).

In summary, also the assessment in 2015 is in line with the view that at least a few genotypes of *F. excelsior* in Feldkirchen show a fairly durable high partial (quantitative) resistance to *H. fraxineus*. Whether the resistance of such clones would be high enough to sustain a more massive infection pressure of the pathogen in a forest environment is unknown. In addition, most studies on genetic resistance of common ash focused on damage in the crown (e.g., McKinney et al. 2014); however, heritability of resistance to root collar lesions and a weak positive correlation between resistance to crown damage and to collar lesions has recently been documented (Muñoz et al. 2016). Resistance to collar infections is impossible to assess in clonal seed orchards because the scions used for grafting and the rootstocks are different genotypes.

It is still discussed whether the trait “early leaf shedding” should be considered as an indication of fungal attack and disease susceptibility, or alternatively, as an avoidance feature because shedding of leaves occurs before the fungus can grow into the shoots. Actually, both phenomena may be relevant, being different from clone to clone (some clones may shed leaves because of the progress of the disease, while others may shed them before the fungus reaches the shoots). In the seed orchards in Feldkirchen and Traföß, there was a significant weakly positive correlation between the mean intensity of leaf shedding in September 2010 and the mean crown dieback intensity in May 2011 (but not at other assessment dates in these seed orchards, and not in Bad Gleichenberg; Freinschlag 2013, Freinschlag and Kirisits 2014). Thus, there was a trend that clones with intensive leaf shedding in September 2010 showed more severe crown dieback in May 2011 than clones with weak leaf shedding. Generally, genotypes with negligible dieback and dense foliage until late in the growing season may be the most resistant or tolerant ones to the disease. A few clones in each of the three seed orchards fulfilled these criteria (e.g., clone no. 14 in Feldkirchen; Figure 1H). Leaf yellowing and senescence, reported as an indicator of resistance to ash dieback in studies in Northern Europe (e.g., McKinney et al. 2014), appears to be of little relevance in Central Europe where *F. excelsior* rarely shows any autumn leaf colouration but leaves are mostly shed when still being green, at the time of the first autumn frosts (Wittmann and Zwisseli 2008, Freinschlag 2013).

Many ash clones in the seed orchards (and also specimens in the field) react to severe infections with vigorous sprouting of shoots from dormant buds, which gives the trees a green appearance despite much shoot damage (Kirisits and Freinschlag 2012, Enderle et al. 2015). However, it is unlikely that such genotypes can sustain the disease in the long-term, and they should thus not be regarded as resistant to *H. fraxineus* (Enderle et al. 2015).

Observations in forests are less encouraging because many initially healthy or only slightly damaged ash trees subsequently decline (Kirisits and Freinschlag 2015). Experience in Grafenegg estate does not indicate any lasting resistance or tolerance. There is certainly variation in the health condition among ash trees, and disease progression varies from year to year (likely influenced by annual weather fluctuations), but it appears that less damaging years do not suffice for substantial recovery of the trees. In Grafenegg, initially more tolerant trees were documented with photographs. However, while the disease progressed slower on these trees, it has nevertheless in the meantime killed them as well. This indicates that the share of trees with durable high resistance in environments where the infection pressure of *H. fraxineus* is intense is likely much lower than the 1 to 3 % frequently mentioned in the literature (Husson et al. 2012, Kjaer et al. 2012, McKinney et al. 2014, Chandelier et al. 2016), if such trees exist at all.

Evolutionary selection in natural ash regeneration

While in the 1990s, there was talk of “ashification” (“Vereschung” in German) of many forest stands in Austria (particularly in the Northern Limestone Alps), meaning vigorous natural rejuvenation of ash being observed frequently, this has now decreased dramatically. Data from Grafenegg indicate a decrease from 800 ha with naturally rejuvenating ash in sufficient numbers in 2004 to only 300 ha in 2012 (and the situation has been worsening since then). Young trees rarely survive to reach tree dimensions; so at the moment there is little evidence for evolutionary selection in ash population due to the disease. Time will tell, but presently the situation is sobering.

Proportion of potentially resistant ash trees

Field observations in Grafenegg and elsewhere in forests as well as in the Austrian seed orchards (Freinschlag 2013, Freinschlag and Kirisits 2014) show that practically all ash trees are to some degree affected by the disease. Kjaer et al. (2012) reported an estimation of one percent of resistant or tolerant trees (i.e., one tree among 101 with a high breeding value for less than 10 % crown damage) based on analyses of progeny trials in Denmark. This cannot be verified for Austria at present, but progeny trials will be initiated soon, which will allow estimations on the share of individuals with a high level of resistance or tolerance to the disease (Kirisits et al. 2016, Unger et al. 2016; see section “*In situ and ex situ conservation, and complementary options*” below). Further investigations will also reveal whether the observation of less than 10 % crown damage in three consecutive years (as in Kjaer et al. 2012) will suffice for assessing durable high resistance. Our experience shows that the disease is progressing even in some initially tolerant trees over the years, at least in forest situations.

Clones for the Austrian seed orchards have originally been selected for their growth vigour as one important criterion (ash dieback was not yet present in the 1980s and 1990s, when selections were made). Consequently, the proportion of clones there with low disease intensity needs to be verified in forest situations. As mentioned, the relatively high share of weakly damaged genotypes in the seed orchards (Figures 1G and 1H; Freinschlag 2013, Freinschlag and Kirisits 2014) is likely because of the rather moderate infection pressure of *H. fraxineus* under the environmental conditions in the orchards.

Ongoing research in Austria

Recently, a conservation and resistance breeding project for *F. excelsior* has been initiated in Austria, “Esche in Not” (“ash in distress”, see http://www.esche-in-not.at/; Kirisits and Cech 2016, Kirisits et al. 2016, Unger et al 2016). It aims at locating healthy and only slightly damaged ash trees in severely affected forest stands (indicative for a high infection pressure of the ash dieback pathogen) across Austria and testing them for disease resistance. Seed is being collected from female and hermaphroditic trees, and a progeny field trial will be initiated in 2015 and 2016 where the seedlings are subjected to natural inoculum of *H. fraxineus*. This shall allow determining narrow-sense heritability of resistance to ash dieback and the breeding values of the mother trees. The mid-term goal is to establish one or several genetically diverse clonal seed orchards with a sufficient number of highly resistant genotypes (selected among mother trees, their progenies and male trees), with the perspective to produce seed sources with high levels of disease resistance or tolerance. In the short term, resistant genotypes shall be propagated via cuttings, to make them available for use in practice. In 2015, seed was collected from 580 trees at 338 localities across Austria (Unger et al. 2016).

Thomas L. Cech of the Austrian Federal Research Centre for Forests (BFW) is involved in two ongoing European projects: “RESIPATH - Responses of European Forests and Society to Invasive Pathogens” (http://www.slu.se/resipath) is funded by BiodivERsA (http://www.biodiversa.org/), a network of funding organisations that are promoting pan-European research, in the form of an ERANET. Ash dieback is one of four target forest pathogens. Within the Horizon 2020 programme of the European Union, “PonTE – Pest Organisms Threatening Europe” (http://www.ponteproject.eu/) also has several target diseases, and it strives to gain further insight into the (molecular) biology of the causative organisms, while using, e.g., remote sensing for surveillance. The project has started work in November 2015.

Master thesis work is currently underway at BFW (A. Wohlmuth; supervision: B. Heinze, BFW and F. Essl, University of Vienna). The idea is to assess paternity among adult trees and saplings in fairly isolated ash stands with molecular markers, and to compare levels of disease in parallel. If trees and saplings of varying health status are present, a correlation test of any resistance among parents and their offspring should be possible.

Management options to mitigate the impact of ash dieback

Silvicultural management of forest stands

Forest stand management does not provide satisfactory immediate “solutions” to mitigate the impact of ash dieback, but aims to diminish the losses due to the disease as best as possible. Previous stand management appears to have had little influence on the mid- to long-term development of ash dieback. For example, the silvicultural system in the Grafenegg estate concentrated on single trees, which were released from competitors early and thus maintained high vitality (Wilhelm et al. 1999, Wilhelm and Rieger 2013). This may have contributed to the comparatively few losses of older trees with broad crowns up to now. However, root collar lesions have been affecting most of these trees recently, and more and more trees fall over.

Besides mortality, growth increment and wood quality losses, the increased hazard risks of diseased trees, due to falling branches and entire trees predisposed by root collar lesions and rot to fall over, are criteria that need to be considered in decision-making. It is recommended that decisions on silvicultural management of stands are based on severity of crown damage and extent of root collar lesions, but not on premature leaf fall (Kirisits 2013, Kirisits and Freinschlag 2014). Later in the growing season, assessment of crown damage is often difficult because of premature leaf shedding; trees for removal should therefore be marked until mid-August at latest. Positive selection of trees with slight crown dieback and which keep their foliage until late in the growing season can also be done later.

Losses are greatest in young, severely damaged pure ash stands where there is no chance that they reach maturity (and thus, to achieve the original goals of forest management). Harvesting of immature trees increases costs. Despite this, clear-cutting of such stands has been practiced as ultimate option by many forest owners in recent years. In mixed-species forests, stand development can possibly be directed towards tree species other than ash. In slightly damaged stands, the original silvicultural goals in terms of target diameters and timber qualities should be followed and their condition assessed on a yearly basis; however, further investments in thinnings and other stand treatments until they show indications of decline are not advisable. Mature ash stands decline at slower rates; here, it is recommended to fell trees at the latest when 70 to 80 % of the crown is dead, in order to recover timber values and avoid losses due to wood discoloration associated with root collar lesions and stem infections via epicormic shoots (Kirisits and Freinschlag 2014). Trees with extensive crown damage and root collar lesions posing a safety risk for forest personnel, visitors, traffic routes and any other infrastructure need to be regularly observed and felled on time (Kirisits and Cech 2016). In mature stands that are not immediately clear-cut, the creation of gaps and understocking are a problem. In floodplain areas, small gaps cannot be planted with the typical light-demanding floodplain hardwood tree species (disease-resistant elms would be much desired for this purpose). Hybrid poplars can be planted in order to fill the gaps in younger stands, in suitable climates. On drier sites outside floodplain areas, silver birch is an option for re-planting gaps.

Alternative tree species

Due to their high susceptibility to ash dieback, planting of *F. excelsior* and *F. angustifolia* is no longer recommended, except for experimental purposes such as progeny trials. In floodplain areas where it is often the main timber species (such as in the Grafenegg estate), replacing ash by other hardwoods is a high-risk option for forest owners that are now suddenly confronted with severely damaged, declining ash stands. It would create a highly irregular age class distribution, and consequently, lead to decades with low yield of valuable timber, as the rotation is typically around 70 years (strong temporal imbalances would be induced). Planting poplars (e.g., 50 % and 50 % hardwoods), with a much shorter rotation of around 25 years, would ease the situation and lower the risk of economic imbalances of the forestry operations. The hardwood species that still present an option for floodplain forests in Austria are maple species, black walnut (*Juglans nigra*), oak (mostly *Quercus robur*) and the London plane tree (*Platanus* x *acerifolia* [syn. *P.*x *hispanica*]). Honey locust (*Gleditschia triacanthos*) may present another option. Non-native tree species are, however, not welcome in Natura 2000 areas, and floodplain forests are included in these nature conservation networks. Silvicultural know-how is also often lacking for such "newcomer" tree species. Furthermore, there may even be presently unknown disease and insect pest problems with some of these. Other tree species such as *Tilia* spp., *Betula pendula*, *Pyrus pyraster* and *Prunus avium* do not tolerate the frequent floods. The non-native *Robinia pseudoacacia*, *Acer negundo* and *Ailanthus altissima*, whose economic value is debatable and which are also viewed critically from the standpoint of nature conservation, are invading naturally.

Some of the above mentioned native species (particularly *A. pseudoplatanus*) as well as black and grey alder (*Alnus glutinosa* and *A. incana*), *Quercus petraea* and European beech are of interest for replacing ash at sites other than floodplain forests. Moreover, forest owners have now started to plant again conifers (mainly *P. abies*, *Larix decidua* and *Pinus sylvestris*) outside their natural ranges.

Non-native ash species will likely be of limited use to replace *F. excelsior* and *F. angustifolia,* not even for forest plantations. Manchurian ash, one fairly tolerant natural host of *H. fraxineus* from Eastern Asia (which, however, displays also symptoms; Drenkhan and Hanso 2010, Drenkhan et al. 2016), is reported to be sensitive to winter and spring frosts, and to grow only to a small tree in Europe (Drenkhan et al. 2014). However, in Central Europe, experience with growing this species is lacking. *F. pennsylvanica* and *F. americana* from North America are to some extent also susceptible to *H. fraxineus*, though less than *F. excelsior* (Drenkhan and Hanso 2010, Gross et al. 2014, McKinney et al. 2014; see also section "*Successive emergence, aetiology and hosts of ash dieback*" above). Despite of these limitations, small scale experimental plantings of some exotic ash species (e.g., *F. mandshurica* and *F. americana*) are presently underway in Eastern Austria, in order to assess their adaptability and growth performance as well as their susceptibility to *H. fraxineus* (Kirisits and Cech 2016).

In situ and *ex situ* conservation, and complementary options

For many years, as in other countries, one key recommendation to practitioners in Austria was to preserve ash trees with unusually low damage levels (putative resistant or tolerant individuals) in all phases of forest stand development, in order to facilitate resistance and thus natural adaption of populations to *H. fraxineus* (e.g., Kirisits 2013a, Kirisits and Freinschlag 2014, 2015, Kirisits and Cech 2016, Kirisits et al. 2016). As mentioned, experience with this practice has been largely unsatisfactory, and it is in most cases no longer justified from an economic point of view. Still, preservation of the very few exceptional trees (with minimal crown damage and no root collar lesions), if any are present, in severely damaged stands makes good sense (Kirisits and Freinschlag 2015, Kirisits and Cech 2016). Of particular interest are vigorous young (thicket- or pole-sized) specimens, as ash dieback progresses more rapidly and is more severe on young trees. Removing all ash trees including also healthy-looking ones bears the risk that potentially resistant genotypes are eliminated forever from the populations. Besides being important for *in situ* conservation, preserved trees which remain in good condition over time provide the basis for *ex situ* conservation measures (see below and section "Ongoing research in Austria" above). Substantial amounts of ash forests in Austria are situated in national parks (e.g., "Kalkalpen National Park", "Gesäuse National Park" and "Donau-Auen National Park") and other areas under nature conservation with minimal human intervention, where the disease "runs its course" unhindered. It remains to be seen how many ash trees will eventually survive repeated infections by *H. fraxineus* in the mid- and long-term in such areas.

There is considerable uncertainty regarding the usefulness of resistance or tolerance in ash against *H. fraxineus*, be it in forestry or amenity plantings. It is therefore worth to think about complementary and alternative strategies for the conservation of this indispensible tree species. Experiences from Dutch elm disease (DED) may help in this respect. Elms have not become extinct completely, mainly because of three factors. First, in Dutch elm disease a vector is required. Elm bark beetles (*Scolytus* spp.) carry the pathogen from tree to tree. When the disease had resulted in a considerable "dilution" of trees across the landscape, occasionally healthy elm trees were found. These trees are unlikely to be resistant but presumably represent "disease escapes", because of decreasing sizes of bark beetle populations and large distances for the beetles to cover to reach host trees (Kirisits 2013b). In a similar way, it may be interesting to find out in the future whether at low densities of ash trees and less available inoculum, the disease can still strike as severely.

A second feature of the elm-DED system is that the beetles prefer older and thus taller trees for maturation feeding, leading to disease escape of many younger specimens (Kirisits 2013b). While this prevents trees from becoming old, some of them still have a change to produce seed before their death. Elms can also form root suckers, and surviving roots allow regrowth. Ash can regrow from coppice, but such sprouts are quickly attacked by *H. fraxineus*, enabling it to grow into and kill ash roots. Coppicing is therefore of little, if any, value to manage ash dieback. As a third factor in elms, resistant trees have been bred in the past, mainly in the form of interspecific hybrids between European and Asian species, but there are also resistant genotypes within the "pure" European species, especially within *U. minor* (Kirisits 2013b, Martin et al. 2015). It may be possible to hybridize the susceptible European ash species, *F. excelsior* and *F. angustifolia*, with Asian ones, particularly with *F. mandshurica* (which also belongs to the section *Fraxinus* within the genus), in order to obtain resistant trees. This is a strategy that has also successfully been followed in American chestnut (*Castanea dentata*) in response to chestnut blight caused by *Cryphonectria parasitica*. It consists of crossing the susceptible *C. dentata* with a resistant species (Chinese or Japanese chestnut), and subsequent backcrossing of resulting hybrids with *C. dentata*; resistant genotypes are continuously selected among the crossings (Prospero and Rigling 2013).

As trees on dry sites seem to be less affected by ash dieback, it may be worthwhile to check whether such sites of "limestone ash" can be suitable for conserving trees, by keeping them alive over many years. "Limestone ash" populations are unlikely to differ genetically from "water ash" (Weiser 1995), although there is some small-scale differentiation across the country (Heinze and Fussi 2016). This means that a large part of the original genetic diversity of the species could be conserved rather easily, without having to take care too much about genetically separate units. The problem with conserving whole stands of ash, however, may be that thereby also higher levels of inoculum will likely be "conserved". The sites may also not be "dry enough" (Willner 1996) and "unfavourable enough" to prevent *H. fraxineus* from killing the ash trees in the long run.

A tradition in German-speaking countries is the planting of single "farmhouse trees" that grow to large, symbolic specimens (Wittmann and Zwisseli 2008). Often well maintained by families over centuries, these trees often stand isolated, next to old, traditional farmhouses, in the surrounding agricultural fields or pastures. Their leaves are usually removed in autumn or largely destroyed during agricultural operations. Old ash trees next to such farmhouses may have some potential for conserving the species, and the planting of young ones could be encouraged. Their isolation may give them a chance to escape the disease, as long as it would be accompanied by good care. Solitary trees in parks, cities, in the urban green and in cultural landscapes may contribute to the conservation of ash in a similar way, but the idea of this suggestion is to keep densities rather low. It could be imagined that the situation of ash may once resemble that of *Ginkgo biloba*, the maidenhair tree, which has only survived in human care (as a classical Chinese and Japanese temple tree) into our days.

Complete evacuation of the species, e.g., to southern European locations outside of the present distribution range of *F. excelsior*, may also be discussed. Mountains in Southern Spain, Southern Italy including Sicily, Southern Greece and Southern Turkey may provide suitable habitats. It is of course difficult to imagine large quantities of ash being exported from disease-affected areas, and creating a disease-free environment for them "in exile" would be a great challenge. A limited amount of plantations may, however, serve the purpose of conservation. Examples of "exiled" tree populations that can be kept free of diseases do exist, e.g., with apple trees (*Malus domestica*) in the mountains in Jordan (Salem et al. 2005) and with *Ulmus* spp. planted for *ex situ* conservation and breeding purposes in areas in Spain where elms do not occur naturally (Kirisits 2013b).

In order to give ash a high chance to survive, it may be necessary to combine all of the strategies discussed above. Resistant or tolerant genotypes should be cultivated or conserved in conditions most favourable to their health and unfavourable for disease development, and possibly be backed up in disease-free areas.

Conclusions

As a Central European country, Austria has been hit heavily by ash dieback since the early years of the millennium. The disease has progressed in the course of very few years to affect practically all ash tree stands in the country. It is now jeopardizing the continued cultivation of the tree as a timber species, affects other ecosystem services, and has numerous negative ecological consequences. Urgent conservation measures are necessary. Hopes lie in the detection and breeding of partially resistant or tolerant genotypes, but it remains to be seen how successful this strategy will be for ash in the forests. As a landscape and shade tree, alternative options are possible and should be considered as well. A combination of all possible measures may be necessary in order to conserve ash in sufficient numbers and thus, to conserve large parts of its genetic diversity. It may well be that our generation is the last one to have known majestic old ash trees and stands of this species in all their genetic and ecological diversity.

Acknowledgements

Research in the BFW lab was financed by the EU-funded research project “RAP – Realising Ash’ Potential” – contract QLK5-CT-2001-00631 of the EC’s Fifth Framework Programme. Research on ash dieback received funding from the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management (BMLFUW research project no. 100343), the provincial governments of Lower Austria, Styria, Upper Austria, Carinthia, Salzburg and Burgenland, the Forest Office and Urban Agriculture (MA 49) of the Vienna City Administration, the Austrian Federal Forests (ÖBf AG), the foundation “120 Jahre Universität für Bodenkultur” as well as the European Union’s Seventh Framework Programme FP7 2007-2013 (KBBE 2009-3) under grant agreement 245268 ISEFOR. The ash conservation project “Bedrohtes Erbgut Esche” (2015-2019) is funded by the Chamber of Agriculture for Austria, the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management (BMLFUW research project no. 101113), the “Österreichischer Forstverein" and the governments of all Austrian provinces. Further funding from the COST Action “*Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management - FRAXBACK” – FP1103 is gratefully acknowledged. Thomas Baschny (Lebensministerium – Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management) provided nursery plant production data, Ilse Strohschneider (BFW) information on the movement of ash plants from EU countries into Austria, and Thomas L. Cech (BFW) information on ongoing projects. The latter and Katharina Schwanda (BFW and IFFF-BOKU) are acknowledged for their assistance in compiling information on ash dieback shown in Table 1.

References

Baschny, T. and Strohschneider, I. 2012. Forstpflanzenbilanz für Österreich von 1991 bis 2011 [Forest plant balance in Austria from 1991 to 2011]. *BFW-Praxisinformation* 29: 9-10 (in German).

Cech. T.L. 2005. Blattkrankheiten und vorzeitiger Laubfall – eine Folge des kühlfeuchten Sommers 2005 [Fungal diseases of leaves – a consequence of the cool and wet summer 2005]. *Forstschutz Aktuell* 34: 11-12 (in German).

Cech, T.L. 2006a. Auffallende Schadfaktoren an Waldbaumen im Jahr 2005 [Striking damaging agents on forest trees in 2005]. *Forstschutz Aktuell* 35: 6-7 (in German).

Cech, T.L. 2006b. Eschenschäden in Österreich [Ash dieback and premature leaf shedding in Austria]. *Forstschutz Aktuell* 37: 18-20 (in German).

Cech, T.L. 2008. Eschenkrankheit in Niederösterreich – neue Untersuchungsergebnisse [Dieback of ash in Lower Austria – new results]. *Forstschutz Aktuell* 43: 24-28 (in German).

Cech, T.L. and Hoyer-Tomiczek U. 2007. Aktuelle Situation des Zurücksterbens der Esche in Österreich [Actual situation of dieback of ash in Austria]. *Forstschutz Aktuell* 40: 8-10 (in German).

Cech, T.L., Keßler, M. and Brandstetter, M. 2012. Monitoring ash dieback in Austria / Monitoring des Zurücksterbens der Esche in Österreich. In: G. Steyrer, C. Tomiczek, G. Hoch and C. Lackner (Editors), Proceedings of the Third Meeting of Forest Protection and Forest Phytosanitary Experts, Federal Research Centre for Forests, Department of Forest Protection, Vienna, Austria, 14–16 October 2009. *Forstschutz Aktuell* 55: 56-58 (in German).

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: 289-297.

Damm, H.J. 1997. Seltene Eschen II [Rare ashes II]. In: WWF Austria (Editor), Proceedings of the symposium "Zukunft für gefährdete Baumarten? Rückbringung und Förderung seltener und gefährdeter Baum- und Straucharten" ["A future for endangered tree species? Restoration and promotion of rare and endangered tree and shrub species"], Vienna, Austria, 1 October 1997: 11-17 (in German).

Drenkhan, R. and Hanso, M. 2010. New host species for *Chalara fraxinea*. *New Disease Reports* 22: 16.

Drenkhan, R., Sander, H. and Hanso, M. 2014. Introduction of Mandshurian ash (*Fraxinus mandshurica* Rupr.) to Estonia: Is it related to the current epidemic on European ash (*F. excelsior* L.)? *European Journal of Forest Research* 133: 769-781.

Drenkhan, R., Solheim, H., Bogacheva, A., Riit, T., Adamson, K., Drenkhan, T., Maaten, T. and Hietala, A.M. 2016. *Hymenoscyphus fraxineus* is a leaf pathogen of local Fraxinus species in the Russian Far East. *Plant Pathology*, in press, doi: 10.111/ppa12588.

Enderle, R., Nakou, A., Thomas, K. and Metzler, B. 2015. Susceptibility of autochthonous German *Fraxinus excelsior* clones to *Hymenoscyphus pseudoalbidus* is genetically determined. *Annals of Forest Science* 72: 183-193.

Freinschlag, C. 2013. Untersuchungen zum Eschentriebsterben in Eschen-Samenplantagen in Österreich [Investigations on ash dieback in ash seed plantations in Austria]. Master thesis, Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), University of Natural Resources and Life Sciences, Vienna (BOKU), Vienna, Austria, 140 pp (in German).

Freinschlag, C. and Kirisits, T. 2014. Untersuchungen zum Eschentriebsterben in Eschen-Samenplantagen in Österreich[Investigations on ash dieback in ash seed plantations in Austria]. In: M. Mitter., M.H. Gerzabek and J. Glößl (Editors), Elektronische Publikation, Präsentation von geförderten Projekten der Stiftung „120 Jahre Universität für Bodenkultur", Universität für Bodenkultur Wien (BOKU), Vienna, 11 March 2014: 25-42 (in German).

Fussi, B., Koziel, A. and Heinze, B. 2008. Selection of seed orchard parents in common ash (*Fraxinus excelsior*): A genetic comparison of seed orchard composition with commercial seed lots (poster). In: D. Lindgren (Editor), Seed orchards – Proceedings from a conference at Umeå, Sweden, September 26-28, 2007 (ed., Dag Lindgren). Umeå (ISBN: 978-91-85911-28-8): 4.

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V. and Sieber, T.N. 2014. *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. *Molecular Plant Pathology* 15(1): 5-21.

Gschwantner, T., Gabler, K., Schadauer, K. and Weiss, P. 2010. Chapter 1 – Austria. In: E. Tomppo, T. Gschwantner, M. Lawrence and R.E. McRoberts (Editors), National Forest Inventories – Pathways for Common Reporting, Netherlands, Springer, 57-71.

Halmschlager, E. and Kirisits, T. 2008. First report of the ash dieback pathogen *Chalara fraxinea* on *Fraxinus excelsior* in Austria. *Plant Pathology* 57(6): 1177.

Heinze, B. 2001. Verbesserung der Produktivität der Esche in Europa [Improvement of the productivity of ash]. *Österreichische Forstzeitung (Vienna)* 112(6): 8-9 (in German).

Heinze, B. 2007. A database of PCR primers for the chloroplast genomes of higher plants. *Plant Methods* 3: 4.

Heinze, B. and Fussi, B. 2016. Pre-disease levels of genetic diversity and differentiation among Austrian ash (*Fraxinus excelsior* L.) seedlots. *Baltic Forestry*, submitted.

Heinze, B., Koziel, A. and Fussi, B. 2005. Wie vermehrt man sich unter Bäumen? Grenzenloser Pollenflug bei Eschen und anderen Baumarten? [How to multiply among trees? Limit-less pollon flow of ash and other tree species?]. Presentation given at Naturschutzbund Wien, Vienna, 22 May 2005 (in German).

Heuertz, M., Fineschi, S., Anzidei, M., Pastorelli, R., Salvini, D., Paule, L., Frascaria-Lacoste, N., Hardy, O.J., Vekemans, X. and Vendramin, G.G. 2004. Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe. *Molecular Ecology* 13(11): 3437-3452.

Heuertz, M., Carnevale, S., Fineschi, S., Sebastiani, F., Hausman, J. F., Paule, L. and Vendramin, G.G. 2006. Chloroplast DNA phylogeography of European ashes, *Fraxinus* sp. (Oleaceae): roles of hybridization and life history traits. *Molecular Ecology* 15(8): 2131-2140.

Husson, C., Caël, O., Grandjean, J.P., Nageleisen, L.M. and Marçais, B. 2012. Occurrence of *Hymenoscyphus pseudoalbidus* on infected ash logs. *Plant Pathology* 61(5): 889-95.

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. *Journal of Agricultural Extension and Rural Development* 4(9): 223-226.

Kirisits, T. 2008. Eschenpathogen *Chalara fraxinea* nun auch in Kärnten nachgewiesen [First report of the ash dieback pathogen *Chalara fraxinea* from Carinthia]. *Forstschutz Aktuell* 45: 28-30 (in German).

Kirisits, T. 2011. Eschentriebsterben in Tirol und Vorarlberg [Ash dieback in Tyrol and Vorarlberg]. *Forstschutz Aktuell* 51: 14-18 (in German).

Kirisits, T. 2013a. Eschentriebsterben: Neue Erkenntnisse und Empfehlungen [Ash dieback: new insights and recommendations]. *Kärntner Forstverein Information (KFV Info) [Klagenfurt, Austria]* 70/January 2013: 12–14 (in German).

Kirisits, T. 2013b. Dutch elm disease and other *Ophiostoma* diseases. In: P. Gonthier and G. Nicolotti (Editors), Infectious forest diseases. CABI, Wallingford, UK, 256-282.

Kirisits, T. 2016. Further observations on the association of *Hymenoscyphus fraxineus* with *Fraxinus ornus*. *Baltic Forestry*, submitted.

Kirisits, T. and Konrad, H. 2004. Dutch elm disease in Austria. *Investigación Agraria: Sistemas y Recursos Forestales (Forest Resources and Systems)* 13(1): 81-92.

Kirisits, T. and Freinschlag, C. 2012. Ash dieback caused by *Hymenoscyphus pseudoalbidus* in a seed plantation of *Fraxinus excelsior* in Austria. *Journal of Agricultural Extension and Rural Development* 4(9): 184-191.

Kirisits, T. and Freinschlag, C. 2014. Eschentriebsterben: Wissensstand und Praxisempfehlungen [Ash dieback: state of knowledge and recommendations for practice]. *Kärntner Forstverein Information (KFV Info) [Klagenfurt, Austria]* 73/January 2014: 18-20 (in German).

Kirisits, T. and Freinschlag, C. 2015. Eschentriebsterben: Gesunde Eschen erhalten und fördern! [Ash dieback: preserve and promote healthy ashes!] *Kärntner Forstverein Information (KFV Info) [Klagenfurt, Austria]* 76/January 2015: 9-11 (in German).

Kirisits, T. and Schwanda, K. 2015. First definite report of natural infection of *Fraxinus ornus* by *Hymenoscyphus fraxineus*. *Forest Pathology* 45(5): 430-432.

Kirisits, T. and Cech, T.L. 2016. Aktueller Wissensstand zum Eschen(trieb)sterben [Current state of knowledge on ash dieback]. In: M. Mitter (Editor), Eschen(trieb)sterben und Verkehrssicherheit [Ash dieback and hazard risks]. Broschüre über die Informationsveranstaltung vom Mittwoch, den 6. April 2016, in Salzburg. Salzburg: Land Salzburg, Abteilung 4 Lebensgrundlagen und Energie, Referat 4/02 - Landesforstdirektion, 7-21 (in German); https://www.salzburg.gv.at/agrarwald_/Documents/Eschentriebsterben.pdf.

Kirisits, T., Matlakova, M., Mottinger-Kroupa, S. and Halmschlager, E. 2008. Verursacht *Chalara fraxinea* das Zurücksterben der Esche in Österreich? [Is *Chalara fraxinea* the causal agent of ash dieback in Austria?] *Forstschutz Aktuell* 43: 29-34 (in German).

Kirisits, T., Matlakova, M., Mottinger-Kroupa, S., Cech, T.L. and Halmschlager, E. 2009. The current situation of ash dieback caused by *Chalara fraxinea* in Austria. In: H.T. Doğmuş-Lehtijärvi (Editor), Proceedings of the Conference of IUFRO Working Party 7.02.02, Eğirdir, Turkey, 11-16 May 2009. *SDU Faculty of Forestry Journal (Isparta), Serial: A, Special Issue*: 97-119.

Kirisits, T., Matlakova, M., Mottinger-Kroupa, S., Halmschlager, E. and Lakatos, F. 2010a. *Chalara fraxinea* associated with dieback of narrow-leafed ash (*Fraxinus angustifolia*). *Plant Pathology* 59(2): 411.

Kirisits, T., Kräutler, K. and Cech, T.L. 2010b. Wieder früher Blattfall der Esche [Again early leaf shedding of ash]. *Forstzeitung (Vienna)* 121(10): 30-31 (in German).

Kirisits, T., Kräutler, K., Keßler, M., Steyrer, G. and Cech, T.L. 2011. Österreichweites Eschentriebsterben [Austria-wide ash dieback]. *Forstzeitung (Vienna)* 122(05): 36-37 (in German).

Kirisits, T., Kritsch, P., Kräutler, K., Matlakova, M. and Halmschlager, E. 2012. Ash dieback associated with *Hymenoscyphus pseudoalbidus* in forest nurseries in Austria. *Journal of Agricultural Extension and Rural Development* 4(9): 230-235.

Kirisits, T., Cech, T.L., Freinschlag, C., Hoch, G., Konrad, H., Unger G.M., Schüler, S. and Geburek, T. 2016. Eschentriebsterben: Wissensstand und Projekt „Esche in Not“ [Ash dieback: state of knowledge and project “ash in distress”]. *Kärntner Forstverein Information (KFV Info) [Klagenfurt, Austria]* 79/January 2016: 32-35 (in German).

Kjaer, E.D., McKinney, L.V., Nielsen, L.R., Hansen, L.N. and Hansen, J.K. 2012. Adaptive potential of ash (*Fraxinus excelsior*) populations against the novel emerging pathogen *Hymenoscyphus pseudoalbidus*. *Evolutionary Applications* 5(3): 219-228.

Kräutler, K. and Kirisits, T. 2012. The ash dieback pathogen *Hymenoscyphus pseudoalbidus* is associated with leaf symptoms on ash species (*Fraxinus* spp.). *Journal of Agricultural Extension and Rural Development* 4(9), 261-265.

Kräutler, K., Treitler, R. and Kirisits, T. 2015. *Hymenoscyphus fraxineus* can directly infect intact current-year shoots of *Fraxinus excelsior* and artificially exposed leaf scars. *Forest Pathology* 45(4): 274-280.

Lenz, H.D., Bartha, B., Straßer, L., Lemme, H. 2016. Development of ash dieback in South-Eastern Germany and the increasing occurrence of secondary pathogens. *Forests* 7(2): 41.

Lexer, C., Heinze, B., Alia, R. and Rieseberg, L.H. 2004. Hybrid zones as a tool for identifying adaptive genetic variation in outbreeding forest trees: lessons from wild annual sunflowers (*Helianthus* spp.). *Forest Ecology and Management* 197(1-3): 49-64.

Madigan, A., Belka, M., Taylor, A.F.S., Kirisits, T., Cleary, M., Nguyen, D., Elfstrand, M. and Woodward, S. 2015. Can *Hymenoscyphus fraxineus* infect hardy members of the Oleaceae other than ash species? *Forest Pathology* 45(5): 426-429.

Martín, J.A., Solla, A., Venturas, M., Collada, C., Domínguez, J., Miranda, E., Fuentes, P., Burón, M., Iglesias, S. and Gil, L. 2015. Seven *Ulmus minor* clones tolerant to *Ophiostoma novo-ulmi* registered as forest reproductive material in Spain. *iForest – Biogeosciences and Forestry* 8, 172-180.

Matlakova, M. 2009. Untersuchungen zum gegenwärtigen Zurücksterben der Eschen [Studies on the current ash dieback]. Master thesis, Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), University of Natural Resources and Life Sciences, Vienna (BOKU), Vienna, Austria, 73 pp (in German).

McKinney, L.V., Nielsen, L.R., Collinge, D.B., Thomsen, I.M., Hansen, J.K. and Kjær, E.D. 2014. The ash dieback crisis: genetic variation in resistance can prove a long-term solution. *Plant Pathology* 63(3): 485-99.

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*, in press.

Pfister, A. 2012.: Aktuelle Schäden durch Eschenbastkäfer in der Steiermark [Current damage by ash bark beetles in Styria]. *Forstschutz Aktuell* 54: 22-25 (in German).

Piribauer, V.-C. 2013. Untersuchungen zum Eschentriebsterben an *Fraxinus angustifolia* und *Fraxinus pennsylvanica* [Investigations on ash dieback on *Fraxinus angustifolia* and *Fraxinus pennsylvanica*]. Master thesis, Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), University of Natural Resources and Life Sciences, Vienna (BOKU), Vienna, Austria, 120 pp. (in German).

Prospero, S. and Rigling, D. 2013. Chestnut blight. In: P. Gonthier and G. Nicolotti (Editors), Infectious Forest Diseases. CABI, Wallingford, UK, 318-339.

Richards, A.J. 1997. Plant Breeding Systems - Second edition. Garland Science/Chapman & Hall, London, 529 pp.

Salem, N., Mansour, A. and Al-Musa, A. 2005. Viruses of pome fruit trees in Jordan. *Journal of Plant Pathology* 87(2): 123-126.

Schwanda, K. and Kirisits, T. 2016. Pathogenicity of *Hymenoscyphus fraxineus* towards leaves of three European ash species: *Fraxinus excelsior*, *F. angustifolia* and *F. ornus*. *Plant Pathology* 65: 1071-1083.

Thomsen, I. M. 2013. Das Eschentriebsterben an Stadt- und Straßenbäumen – eine Situationsbeschreibung aus Dänemark [Ash dieback of urban and rural trees – a status from Denmark]. *Jahrbuch der Baumpflege 2014*: 103-109 (in German).

Timmermann, V., Børja, I., Hietala, A.M., Kirisits, T. and Solheim, H. 2011. Ash dieback: pathogen spread and diurnal patterns of ascospore dispersal, with special emphasis on Norway. *EPPO Bulletin* 41(1): 14-20.

Treitler, R. 2014. Susceptibility of three *Fraxinus* species to the ash dieback pathogen *Hymenoscyphus fraxineus*. Master thesis, Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), University of Natural Resources and Life Sciences, Vienna (BOKU), Vienna, Austria, 107 pp..

Unger, G.M., Konrad, H., Schüler, S., Geburek, T. and Kirisits, T. 2016. Züchtungs- und Erhaltungsinitiative „Esche in Not“ [Resistance breeding and conservation initiative “ash in distress”]. *Forstzeitung (Vienna)* 127(4): 12-13 (in German).

Weiser, F. 1995. Beitrag zur Existenz von Ökotypen bei gemeiner Esche (*Fraxinus excelsior* L.) [Contribution to the existence of ecotypes in common ash (*Fraxinus excelsior* L.)]. *Forstarchiv* 66(6): 251-257 (in German).

Wilhelm, G.J., Letter, H.-A. and Eder, W. 1999. Konzeption einer naturnahen Erzeugung von Wertholz [Concept of a close-to-nature production of quality timber]. *Allgemeine Forst Zeitschrift/Der Wald* 1999(5): 232-240 (in German).

Wilhelm, G.J. and Rieger, H. 2013. Naturnahe Waldwirtschaft mit der QD-Strategie [Close-to-nature silviculture with the QD strategy]. Ulmer, Stuttgart, 207 pp (in German).

Willner, W. 1996. Die Gipfeleschenwälder des Wienerwaldes [The hilltop ash forests of the Vienna Woods]. *Verhandlungen der Zoologisch-Botanischen Gesellschaft Österreich* 133: 133-184 (in German).

Wittmann, R. and Zwisseli, J. 2008. Hofbäume – Tradition, Baumarten, Pflege [Farmhouse trees – tradition, tree species, care]. Ulmer, Stuttgart, 144 pp. (in German).

Zukrigl, K. 1997. Seltene Eschen I [Rare ash species I]. In: WWF Austria (Editor), Proceedings of the symposium “Zukunft für gefährdete Baumarten? Rückbringung und Förderung seltener und gefährdeter Baum- und Straucharten” [“A future for endangered tree species? Restoration and promotion of rare and endangered tree and shrub species”], Vienna, Austria, 1 October 1997: 7-10 (in German).

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 53 – 60.

Ash dieback in Wallonia, southern Belgium: research on disease development, resistance and management options

A. CHANDELIER[1*], L. DELAHAYE[2], H. CLAESSENS[3], L. LASSOIS[3]

[1]Walloon Agricultural Research Centre (CRA-W), Life Science Department, B-5030 Gembloux, Belgium; [2]Walloon Region, Department Natural Ecosystem and Agriculture, B-5030 Gembloux, Belgium; [3]University of Liège, Gembloux Agro-Bio Tech, BIOSystem Engineering (BIOSE) Department, Forest Resources Management, B-5030 Gembloux, Belgium.
** corresponding author: a.chandelier@cra.wallonie.be*

1. Introduction

1.1. Importance of ash

The Walloon forest covers 556,200 ha, representing 33% of the territory of Wallonia. In terms of timber production, common ash (*Fraxinus excelsior*), the only ash species present in the Walloon forest, is the third most important hardwood species, after native oaks and beech. It is present on about 45,000 ha of the 269,900 ha covered by the hardwood stands, in pure (8,700 ha) or mixed (36,300 ha) forests. The forests of ash trees only belong mainly to private owners (71%) and most of the trees are non-mature (H. Lecomte 2015 – Inventaire Permanent des Ressources Forestières de Wallonie). As shown in Fig. 1, ash trees are not evenly distributed in Wallonia. They are more common in the eco-regions of ‘Condroz’, ‘Région limoneuse’ and ‘Lorraine’, where high levels of soil nutrients and water content provide optimal growth conditions for this resource-demanding species (Alderweireld et al. 2015; Claessens et al. 1994). Ash is also often used as a roadside tree and in hedgerows.

1.2. Ash dieback and its spread (disease history)

Ash dieback caused by *Hymenoscyphus fraxineus* was first reported in Poland in 2006 (Kowalski, 2006). This fungus is an invasive pathogen (Husson et al. 2011) that originates from Asia (Baral et al. 2014). Over the past 10 years, the pathogen has spread rapidly within Europe, causing a massive decline in the number of ash trees of all ages (Gross et al. 2014; Timmermann et al. 2011).

1.2.1. Methodology of survey

In 2008, Walloon forest owners and nurserymen were informed of this new phytosanitary threat through the publication of documents describing the disease symptoms (Chandelier 2008a; Chandelier 2008b).

From 2009 to 2011, a survey seeking to detect the pathogen was conducted each year in Wallonia. The survey units were limited to 0.5 ha and each unit contained a minimum of 10 ash trees. Visits were conducted each year from June to September, with a complete inventory being taken of each unit. The observations focused on the presence of wilted leaves still attached to branches and of cankers on ash regeneration, on coppice shoots from felled ash trees and on epicormic shoots on the tree trunks. All samples displaying symptoms that could be caused by the pathogen were analyzed using real-time polymerase chain reaction (PCR), following the procedure developed by Chandelier et al. (2010) and isolated on Malt Extract Agar (MEA) supplemented with an antibiotic, as described by Kowalski (2006). For samples revealed as positive in the molecular test, the Internal Transcribed Spacer Region (ITS) was sequenced on DNA extracted from mycelium of pure culture, following EPPO protocol (2013), in order to confirm the results.

1.2.2. Results of the survey

In 2009, 65 survey units were established, 31 of which were forest stands with ash trees and 34 comprised roadside ash trees along the borders with France, Luxembourg and Germany (Fig. 1a). Samples were collected from branches showing lesions on the bark in 45 of the 65 units. Real-time PCR showed all these samples to be negative. Isolation on MEA revealed the presence of other pathogenic fungi, including *Diplodia* sp., *Phoma* sp. and *Phomopsis* sp.. In 2010, the survey was extended to 204 survey units comprising those set up in 2009 and 139 additional plots, located mainly in the forest area. The pathogen was isolated on ash regeneration in 12 of the 204 survey units (~6%), among which were six plots surveyed in 2009 (Fig. 1b). The identity of the pathogen was confirmed by sequencing the ITS region (Chandelier et al. 2011). In 2011, 86 survey units were selected in the forest, 15 of which had already been monitored in 2009 and 2010. The pathogen was detected in 55 of the 86 survey units (~64%) (Fig. 1c). The pathogen was detected in nine of the units surveyed in 2009 and 2010. These results show a very rapid progression of the disease in Wallonia in only 2 years (2010-2011).

In 2012, forest managers were trained to identify the symptoms of the disease and were asked to send symptomatic samples to the Walloon Agricultural Research Centre (CRA-W) laboratory. Samples from 80 forest stands that had not been monitored in 2009, 2010 or 2011 were analyzed. The disease was present in 100% of the surveyed forest stands (Fig. 1d).

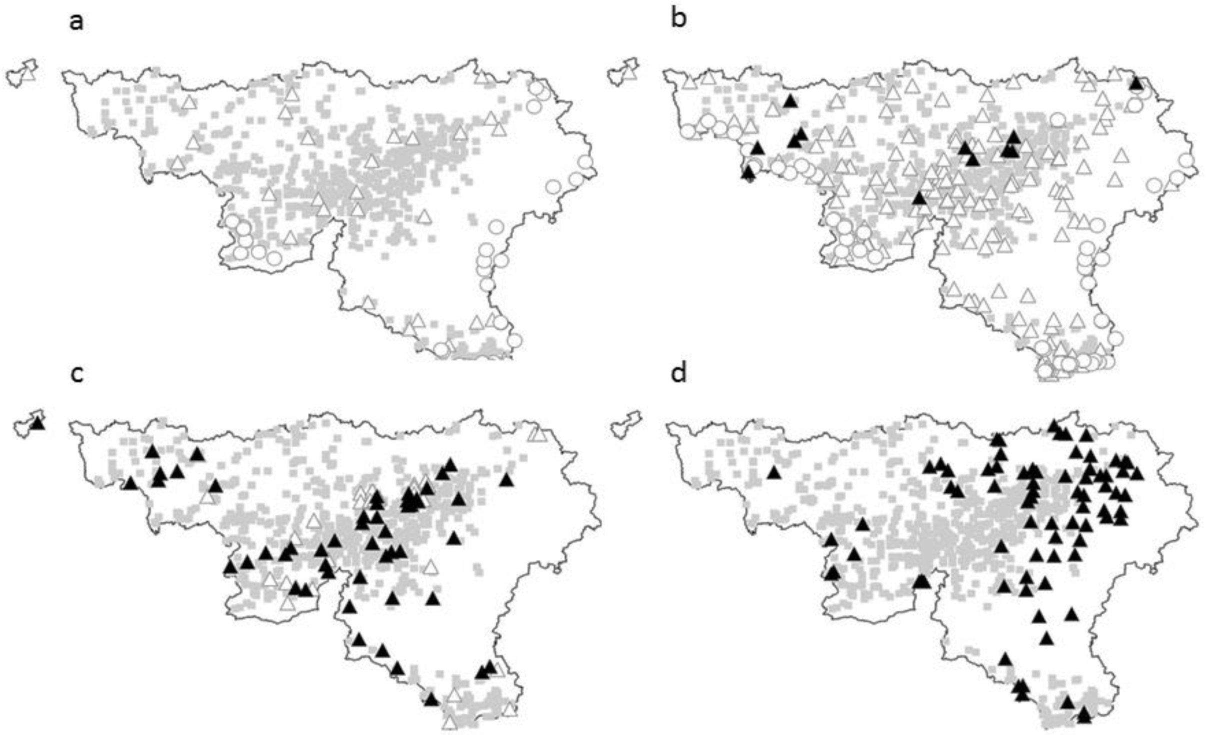


Figure 1 Distribution of *Fraxinus excelsior* (grey squares) and monitoring carried out in Wallonia in **(a)** 2009, **(b)** 2010, **(c)** 2011 for ash dieback caused by *Hymenoscyphus fraxineus*. In 2012 **(d)**, samples were collected by forest owners. Survey units were set up in forests (triangles) or along roads (dots). Black symbols indicate ash dieback-affected plots.

Figure 2 Collar lesions on trees in ash dieback-affected stands. Photo: A. Chandelier.

2. Research on ash dieback

2.1. Development and impact of ash dieback

In 2012, discrete lesions forming a triangle at the collar were observed on a few ash trees originating from stands where the disease had first been reported in 2010 (Fig. 2). A study carried out in France demonstrated that this symptom was frequent in ash dieback-affected stands and caused by *H. fraxineus* (Husson et al. 2012).

In order to evaluate the prevalence and progression of collar lesions in ash dieback-affected stands of various ages, in 2013 a survey was initiated of 268 ash trees originating from 17 forest stands (pure and mixed forests) evenly distributed in the area with ash stands in Wallonia. Observations were carried out in June each year. As shown in Table 1, the overall proportion of trees with collar lesions greatly increased over the years, reaching 69% of the surveyed trees in June 2015. The rate of progression differed from one stand to another. The first dead trees appeared in 2014 in the stand where collar lesions had first been detected in 2012 and the number of sites with dead trees increased in 2015. A statistical analysis carried out on data from 2013 and 2014 did not reveal any significant link between a tree's diameter-at-breast height (DBH) and collar lesion occurrence (Chandelier et al. 2016).

Table 1 Evolution of the number of ash trees with collar lesions in 17 ash stands in Wallonia over 3 years (measurements carried out in June 2013, June 2014 and June 2015). In brackets = dead trees; DBH=diameter-at-breast height.

#	Location	forest type	Mean DBH[1]	Year of infection	N	2013[2]	2014[2]	2015
1	Hainin	pure	17.2 ± 3.6	2010	18	16 (0)	16 (3)	17 (7)
2	La Calamine	pure	20.2 ± 2.8	2010	20	17 (0)	20 (0)	20 (1)
3	Modave	pure	29.4 ± 6.1	2010	20	17 (0)	18 (0)	18 (1)
4	St Lambert	pure	19.2 ± 2.5	2011	20	7 (0)	12 (0)	18 (1)
5	Minières	pure	45.6 ± 9.2	2011	10	0 (0)	9 (0)	10 (0)
6	Les Halleux	pure	15.7 ± 2.1	2011	15	0 (0)	0 (0)	6 (0)
7	Beloeil	mixed	39.8 ± 8.0	2011	15	3 (0)	4 (0)	8 (0)
8	Rance	pure	18.7 ± 3.2	2011	15	2 (0)	2 (0)	9 (0)
9	Gochenée	pure	34.0 ± 5.9	2011	15	5 (0)	10 (0)	10 (0)
10	Spa	pure	29.7 ± 3.0	2011	15	3 (0)	13 (0)	15 (0)
11	Gesves	mixed	33.7 ± 7.3	2011	15	1 (0)	6 (0)	8 (0)
12	Devant-Les-Bois	mixed	38.7 ± 14.6	2011	15	1 (0)	3 (0)	6 (0)
13	Seraing	pure	23.9 ± 2.4	2011	15	0 (0)	0 (0)	0 (0)
14	Senseruth	mixed	22.9 ± 2.2	2012	15	8 (0)	12 (0)	13 (0)
15	Buzenole	pure	38.6 ± 7.8	2012	15	0 (0)	3 (0)	7 (0)
16	Musson	mixed	41.5 ± 10.8	2012	15	1 (0)	7 (0)	10 (0)
17	Bertrix	pure	15.8 ± 2.1	2012	15	3 (0)	9 (0)	9 (0)
Total					268	84 (0)	144 (3)	184 (10)

[1] measuments carried out in June 2013

[2] already published (Chandelier et al. 2016)

Armillaria spp. have been detected on most of the necrotic tissues at the base of ash trees (Bakys et al. 2011; Enderle et al. 2013; Husson et al. 2012). In order to determine their role in the decline of ash trees, wood and bark samples were collected from the collar lesions of 103 ash trees originating from the 12 ash dieback-affected stands in 2014. Molecular tests were carried out on DNA extracted from these samples in order to detect *H. fraxineus* (Chandelier et al. 2010) and/or *Armillaria* spp. (Lochman et al. 2004). Most of the samples (98%) were infected by *H. fraxineus*. The species *Armillaria gallica* and *A. cepistipes* were also detected in 41% of the samples, but almost systematically in combination with *H. fraxineus*, demonstrating their secondary role in the deterioration of the health status of ash trees affected by *H. fraxineus* (Chandelier et al. 2016). These results suggest that the population of common ash trees will be strongly affected in the future regardless of tree age and that the timber quality might be damaged due to the activity of *Armillaria* species.

From an economic point of view, the harvested volume of ash timber increased greatly in 2015 compared with the 2009-2014 period (Fig. 3A) (Sami Ben Mena, Walloon Region, pers. comm.; ash timber sales from public forests). This situation could result from the degradation of the health status of ash in 2014, which prompted forest owners to cut down a lot of diseased trees. In parallel, the sales price of ash timber (standing trees in the three categories of DBH corresponding to mature trees with a DBH of > 50 cm) fell in 2015 after a period of a slightly rising trend (Fig. 3B) (data from La Fédération Nationale des Experts Forestiers, published in Silva Belgica 2009-2015). All these events indicate that an economic depreciation of ash timber could occur in the near future.

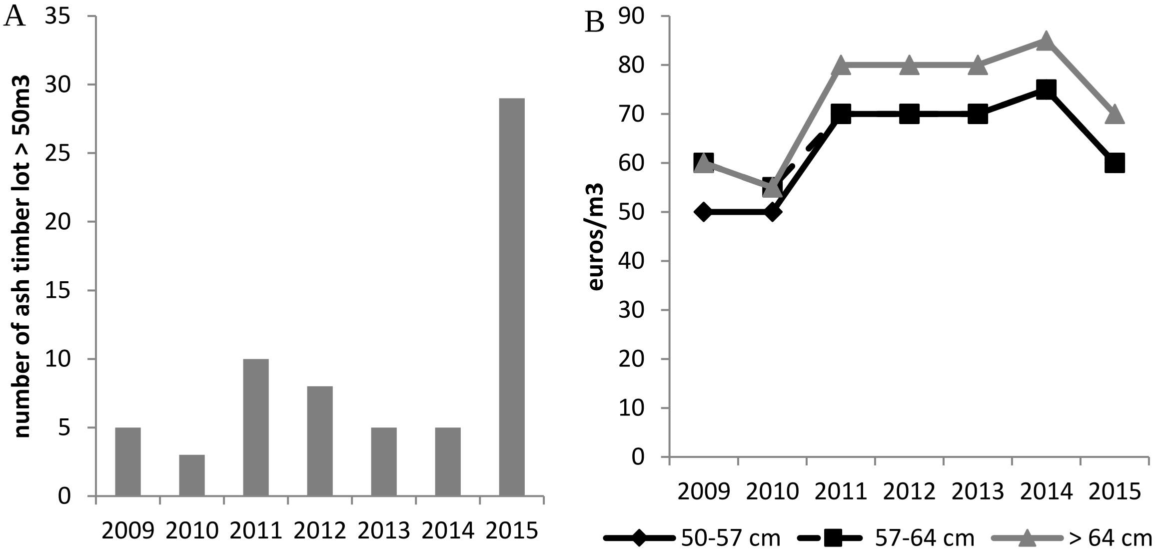


Figure 3 Number of ash timber lots greater than 50 m^3 put on the market in public forests of Wallonia **(A)** and minimum ash wood timber sales price for standing ash trees in three DBH categories in Belgium **(B)** between 2009 and 2015.

2.2. Genetics of ash / population and resistance

Studies conducted in various European countries have shown that susceptibility to ash dieback is genetically determined (e.g., Enderle et al. 2015; Kirisits and Freinschlag 2012; Kjaer et al. 2012; Lobo et al. 2014; McKinney et al. 2011; Pliura et al. 2011; Stener 2013). The natural genetic partial resistance in the ash population documented in these studies provides some hope for the species' conservation and the possibility of breeding for resistance against *H. fraxineus*. In order to be effective, however, genetic control requires maintaining high genetic diversity within and among populations of ash trees and understanding the molecular mechanisms involved in ash tree reaction to pathogen pressure. Maintaining good diversity is essential for the adaptation to current and unknown future local selection pressures.

There are two main projects being implemented in Wallonia aimed at gaining a better understanding of the genetic component of ash dieback susceptibility. The first one is on the quantitative genetics of variation in susceptibility among common ash trees over a period of years. This evaluation is based on identical progeny trials established at various sites before the onset of the disease (1987). It will allow the level of damage due to infection over time to be evaluated, the relevant genetic parameters to be quantified and interesting highly tolerant genotypes to be identified. Despite the problems caused by ash dieback, the molecular mechanisms underlying these quantitative host-pathogen relationships are still unknown. The second project is being conducted in order to gain an insight into these mechanisms by looking at two complementary molecular approaches based on transcriptome and methylome studies. The transcriptomic analyses will identify genes and molecular pathways that govern susceptibility to the disease. The epigenomic approach will focus on cytosine methylation, a heritable modification of DNA that does not involve a change in the DNA sequence. This epigenomic approach is more innovative, especially for forest tree species in which natural variation in epigenetic marks and the relationship with phenotypic traits remains an under-researched area. There is now mounting evidence that heritable variations in relevant traits can be generated through epigenetic mechanisms. Epigenetic variation contributes to the phenotypic plasticity and adaptive capacity of plant species, and could be particularly important for long-lived organisms such as forest trees, where changes in allele frequency are thought to occur very slowly. All this information will be useful and relevant in identifying new sources of adaptive traits in tree breeding for conservation/management under rapid climate change and within the context of increasing pest pressure on forest trees.

3. Management options for mitigating the impact of ash dieback

3.1. Silvicultural management options

In order to meet the demands of forest owners, limit massive felling of ash trees in forests and avoid a drastic decline in ash timber sales prices, guidelines on the management of the disease were proposed in 2015 (Delahaye et al. 2015). As a first step, the health status, tree species composition and development stage of the stand should be evaluated. Then, on the basis of this evaluation, various tree felling options are proposed depending on the tree health status (Table 2). These management guidelines have three objectives: (1) to preserve trees potentially tolerant of the disease and to develop, through natural regeneration, a population of ash trees less susceptible to the disease; (2) to promote tree species diversity and (3) to help forest owners by securing viable economic returns with high-value ash timber.

When establishing new forest stands, ash trees are no longer recommended. Notices have been sent to forest nurseries to encourage them to stop producing ash trees.

Table 2 Decision scheme for the management of ash dieback in forest stands.

Stand characteristics			Tree health status		
Development stage	Tree composition	Health status	Ash with collar lesion	Ash with defoliation > 50%	Ash without symptom and other valuable tree species
Small to medium trees (DBH<50 cm)	Pure	> 33% of symptomatic trees*	Thinning among trees as soon as possible	Thinning among trees as soon as possible	To promote with crop tree silviculture
		< 33% of symptomatic trees*	Felling every tree as soon as possible	Felling every tree as soon as possible	
	Mixed	Not taken into account	Felling every tree as soon as possible	No action	
Large trees (DBH>50cm)	Not taken into account	Tree without collar lesion	-	Thinning as usual	To promote by stand thining
		Tree with collar lesion	Felling every trees within 3 years	Normal thinning within 3 years	

*collar lesion or defoliation > 50%

3.2. Alternative tree species

Ash is one of the most valuable species in the Belgian forest, providing high quality wood on high fertility sites. It plays many roles in forest ecosystems (e.g., soil structuration, nutriment cycle, biodiversity support) (Claessens et al. 1999; Dobrowolska et al. 2008). With regard to the high fertility of ash tree sites, many other tree species can also grow on these sites, but only a few of them have the same ecological, silvicultural and economic impact. Alternative species could include maple species (*Acer pseudoplatanus* and *A. platanoïdes*), which are valuable broadleaved trees that also provide high quality wood (Claessens et al. 1999; Hein et al. 2009), and black alder (*Alnus glutinosa*) (Claessens et al. 2010), especially on humid soils and riverbanks strips

(Debruxelles et al. 2008). The damage caused by the pathogen *Phytophthora x alni* in Belgium (De Merlier et al. 2005), however, limits the use of black alder species along rivers. Silver birch (*Betula pendula*) is another species that, given its colonizing behavior, could replace ash in the recolonization of forest gaps and improved site quality (e.g., microclimate, soil, biodiversity) (Hemery et al. 2010). This species has good development potential within the context of global change (Hemery et al. 2010; Hynynen et al. 2010).

4. Conclusions

Common ash (*Fraxinus excelsior*) is the only species of ash found in the Belgian forest. Its positive impact on forest ecosystem functioning, its capacity for natural regeneration, its high productivity level and the absence of major health problems prior to the introduction of *H. fraxineus* in Europe made it an attractive species for forest managers wanting to produce valuable wood using a species that had a positive ecological impact on the forest ecosystem (Claessens et al. 1994).

The first ash trees suffering from ash dieback were observed in 2010 in areas far from the borders with Germany and France, where the disease was reported in 2008 and 2009, respectively. This suggests that the pathogen did not enter Belgium from the east, but from the north or through infected nursery plants. From 2010 onwards, the progression rate of the disease was rapid and most forest stands with ash were infected within 2 years. This rapid spread is probably linked to the capacity of the fungus to produce a large amount of ascospores in just 3 or 4 months (Chandelier et al. 2014).

In 2012, the first cases of collar lesions caused by *H. fraxineus* were observed on ash trees in stands that had been infected a few years earlier. The proportion of trees with this symptom has increased rapidly since then in the forest stands. The presence of *Armillaria* species has been confirmed in the wood of trees suffering from collar lesions caused by *H. fraxineus* in several cases, suggesting an acceleration of ash degradation in a near future and a decline in timber quality.

Silvicultural management measures have been proposed to help forest managers limit economic losses and encourage the emergence of a population of ash trees that are more tolerant of the disease. These measures, however, will not solve the problem completely. Other woody species have been proposed for reforestation, among which maple (*Acer pseudoplatanus*, *A. platanoïdes*) and silver birch (*Betula pendula)* are the most promising. In the longer term, given the importance of ash in forest management, it would be useful to promote the development of resistant material. For this reason, various Belgian projects are being implemented that are aimed at characterizing the ash population and identifying potentially resistant trees.

Acknowledgements

The authors wish to thank the Wallonia Public Service (SPW), General Operational Directorate DGO3 (Agriculture, Natural Resources and Environment).

References

Alderweireld, M., Burnay, F., Pitchugin, P., Lecomte, H. 2015. Inventaire forestier wallon – Recherches 1994-2012. SPW, DGO3, Direction des Ressources Forestières, Jambes, 236 pp.

Bakys, R., Vasiliauskas, A., Ihrmark, K., Stenlid, J., Menkis, A. and 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.

Baral, H.O., Queloz, V. and Hosoya, T. 2014. *Hymenoscyphus fraxineus*, the correct scientific name for the fungus causing ash dieback in Europe. *IMA Fungus* 5: 79-80.

Chandelier, A. 2008a. Le frêne, une essence menacée en Europe. *Silva Belgica* 115: 28-31.

Chandelier, A. 2008b. *Chalara fraxinea*, un nouveau champignon qui attaque le frêne en Europe : appel à signalement. *Revue de la Fédération wallonne horticole* 50 :1.

Chandelier, A., André, F. and Laurent, F. 2010. Detection of *Chalara fraxinea* in common ash (*Fraxinus excelsior*) using real time PCR. *Forest Pathology* 40: 87-95.

Chandelier, A., Delhaye, N. and Helson, M. 2011. First report of the ash dieback pathogen *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*) on *Fraxinus excelsior* in Belgium. *Plant Disease* 95: 220.

Chandelier, A., Gerarts, F., San Martin, G., Herman, M. and Delahaye, L. 2016. Temporal evolution of collar lesion associated with ash dieback and the occurrence of *Armillaria* in Belgian forests. *Forest Pathology* doi: 10.1111/efp.12258.

Chandelier, A., Helson, M., Dvorak, M. and Gischer, F. 2014. Detection and quantification of airborne inoculum of *Hymenoscyphus pseudoalbidus* using real-time PCR assays. *Plant Pathology* 63: 1296-1305.

Claessens, H., Pauwels, D., Thibaut, A. and Rondeux, J. 1999. Site index curves and autoecology of ash (*Fraxinus excelsior* L.), sycamore (*Acer pseudoplatanus* L.) and cherry (*Prunus avium* L.) in Wallonia (Southern Belgium). *Forestry* 72(3): 171-182.

Claessens, H., Oosterbaan, A., Savill, P. and Rondeux, J. 2010. A review of the characteristics of black alder (*Alnus glutinosa* (L.) Gaertn.) and their implications for silvicultural practices. *Forestry* 83(2): 163-175.

Claessens, H., Thibaut, A., Lecomte, H., Delecour, F., Rondeux, J. and Thill, A. 1994. Le frêne en Condroz, stations et productivité potentielles. Faculté des Sciences Agronomiques de Gembloux (ed), 119 pp.

Debruxelles, N., Claessens, H., Lejeune, P. and Rondeux, J. 2008. Design of a watercourse and riparian strip monitoring system for environmental management. *Environmental Monitoring & Assessment* doi: 10.1007/s10661-008-0496-y.

Delahaye, L., Herman, M., Chandelier, A., Claessens, H. and Marchal, D. 2015. Note de référence pour la gestion du frêne dans le contexte de la crise liée à la chalarose. *Forêt wallonne* 136: 53-58.

De Merlier, D., Chandelier, A., Debruxelles, N., Noldus, M., Laurent, F., Dufays, E., Claessens, H. and Cavelier, M. 2005. Characterisation of alder *Phytophthora* isolates from Wallonia and development of SCAR primers for their specific detection. *Journal of Phytopathology* 155: 99-107.

Dobrowolska, D., Hein, S., Oosterbaan, A., Wagner, S., Clark, J. and Skovsgaard, J.P. 2011. A review of European ash (*Fraxinus excelsior* L.): implications for silviculture. *Forestry* 84(2): 133-148.

Enderle, R., Nakou, A., Thomas, K. and Metzler, B. 2015. Susceptibility of autochthonous German *Fraxinus excelsior* clones to *Hymenoscyphus pseudoalbidus* is genetically determined. *Annals of Forest Science* 72: 183-193.

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.

EPPO 2013. PM7/117 (1). *Hymenoscuphus pseudoalbidus*. *EPPO Bulletin* 43: 449-461.

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V. and Sieber, T.N. 2014: *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. *Molecular Plant Pathology* 15: 5-21.

Hein, S., Collet, C., Ammer, C., Goff, N.L., Skovsgaard, J.P. and Savill, P. 2009. A review of growth and stand dynamics of *Acer pseudoplatanus* L. in Europe: implications for silviculture. *Forestry* 82(4): 361-385.

Hemery, G. E., Clark, J. R., Aldinger, E., Claessens, H., Malvolti, M. E., O'Connor, E., Raftoyannis, Y., Savill, P. S. and Brus, R. 2010. Growing scattered broadleaved tree species in Europe in a changing climate: a review of risks and opportunities. *Forestry* 83(1): 65-81

Hynynen, J., Niemistö, P., Viherä-Aarnio, A., Brunner, A., Hein, S. and Velling, P. 2010. Silviculture of birch (*Betula pendula* Roth and *Betula pubescens* Ehrh.) in northern Europe *Forestry* 83(1): 103-119.

Husson, C., Caël, O., Grandjean, J.P., Nageleisen, L.M. and Marçais, B. 2012. Occurrence of *Hymenoscyphus pseudoalbidus* on infected ash logs. *Plant Pathology* 61: 889-895.

Husson, C., Scala, B., Cael, O., Frey, P., Feau, N., Ioos, R. and Marçais, B. 2011: *Chalara fraxinea* is an invasive pathogen in France. *European Journal of Plant Pathology* 130: 311-324.

Kirisits, T. and Freinschlag, C. 2012. Ash dieback caused by *Hymenoscyphus pseudoalbidus* in a seed plantation of *Fraxinus excelsior* in Austria. *Journal of Agricultural Extension and Rural Development* 4: 184-191.

Kjaer, ED., McKinney, LV., Nielsen, LR., Hansen, LN. and Hansen, JK. 2012. Adaptive potential of ash (*Fraxinus excelsior*) populations against the novel emerging pathogen *Hymenoscyphus pseudoalbidus*. *Evolutionary Applications* 5: 219-228.

Kowalski, T. 2006. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelcior*) in Poland. *Forest Pathology* 36: 264-270.

Lobo, A., Hansen, J.K., McKinney, LV., Nielsen, LR. and Kjaer, ED. 2014. Genetic variation in dieback resistance: growth and survival of *Fraxinus excelsior* under the influence of *Hymenoscyphus pseudoalbidus*. *Scandanivian Journal of Forest Research* 29: 519-526.

Lochman, J., Sery, O. and Mikes, V. 2004. The rapid identification of European *Armillaria* species from soil samples by nested PCR. *FEMS Microbiology Letters* 237, 105-110.

McKinney, LV., Nielsen, LR., Hansen, JK. and Kjaer, ED. 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (*Oleraceae*) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. *Heredity* 106: 788-797.

Pliura, A., Lygis, V., Suchockas, V. and Bartkevicius, E. 2011. Performance of twenty four European *Fraxinus excelsior* populations in three Lithuanian progeny trials with a special amphasis on resistance to *Chalara fraxinea*. *Baltic Forestry* 17(1): 17-34.

Stener, LG. 2013. Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* in southern Sweden. *Scandanivian Journal of Forest Research* 28: 205-216.

Timmermann, V., Børja, I., Hietala, A.M., Kirisits, T. and Solheim, H. 2011: Ash dieback: pathogen spread and diurnal patterns of ascospores dispersal, with special emphasis on Norway. *EPPO Bulletin* 41: 1-7.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 61 – 67.

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

G. SIOEN*, P. ROSKAMS, B. DE CUYPER, M. STEENACKERS

Research Institute for Nature and Forest (INBO), Kliniekstraat 25, B-1070 Brussel
**Corresponding author: geert.sioen@inbo.be*

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.

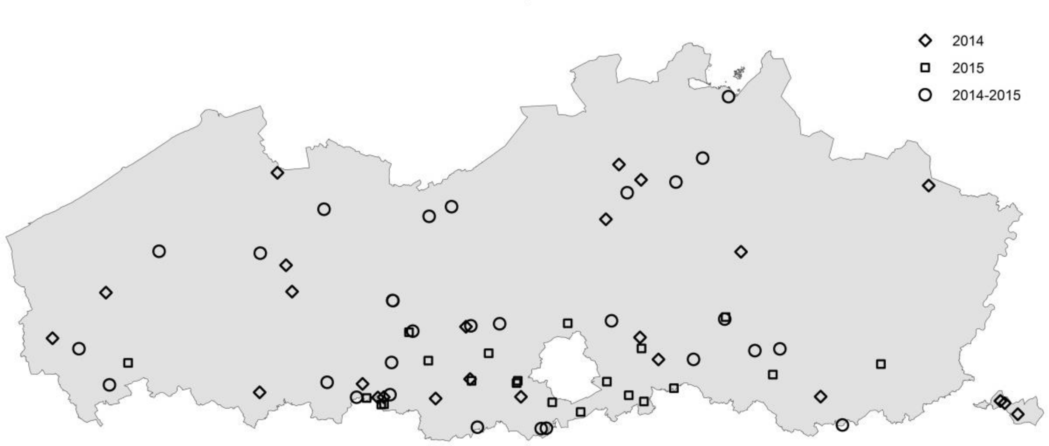


Figure 1 Monitoring plots in Flanders (2014-2015)

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

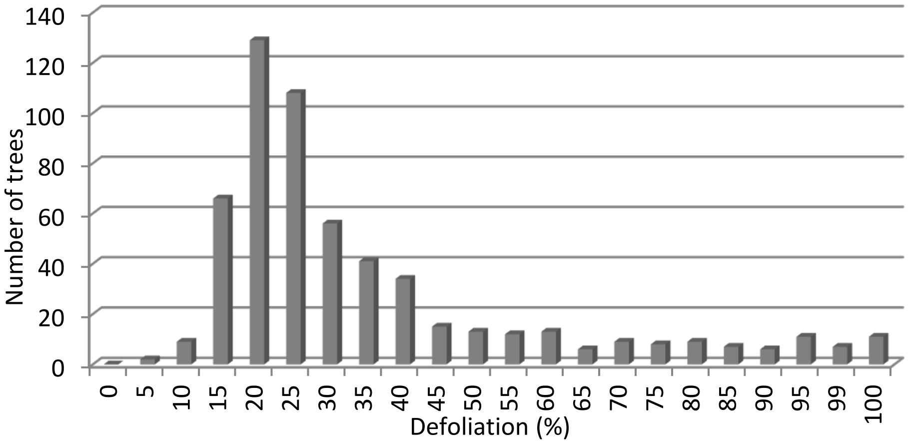


Figure 3 Defoliation of the sample trees in 5 % classes in 2014-2015.

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.

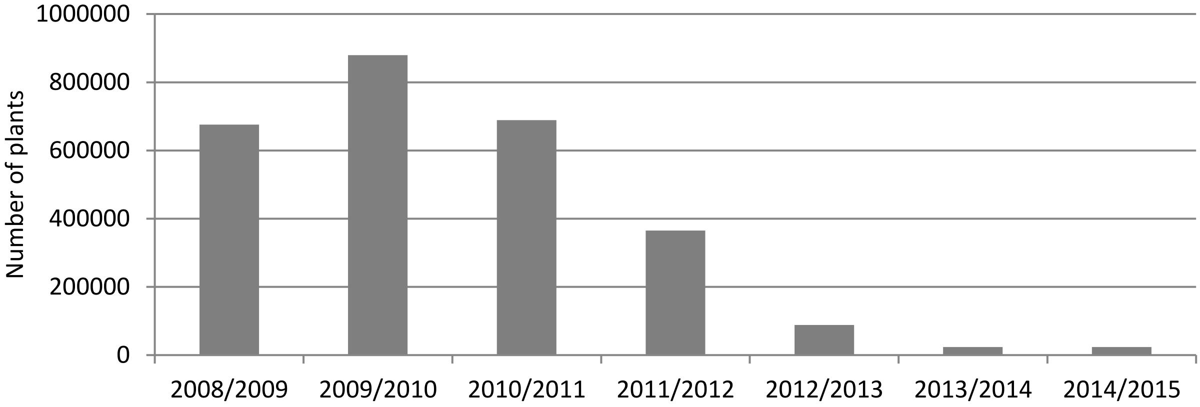


Figure 4 Trade of certified planting material of *Fraxinus excelsior* in Flanders.

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.

References

Cornelis, J., Hermy, M., Roelandt, B., De Keersmaeker, L. and Vandekerkhove, K. 2009. Bosplantengemeenschappen in Vlaanderen, een typologie van bossen gebaseerd op de kruidlaag. Agentschap voor Natuur en Bos en Instituut voor Natuur- en Bosonderzoek, Brussel, INBO.M.2009.5, 316 pp. (in Dutch)

Decleer, K. 2007. Europees beschermde natuur in Vlaanderen en het Belgisch deel van de Noordzee. Habitattypen/Dier- en plantensoorten. Mededelingen van het Instituut voor Natuur en Bosonderzoek, Brussel, INBO.M.2007.01, 584 pp. (in Dutch)

De Haeck, A., Roskams, P., Steenackers, M. and Hens, M. 2013. Advies betreffende de verspreiding van de essenziekte in Vlaanderen. Instituut voor Natuur- en Bosonderzoek, Brussel, INBO.A.2013.82 (in Dutch)

Douglas, G. C., Pliura, A., Dufour, J., Mertens, P., Jacques, D., Fernandez-Manjares, J., Buiteveld, J., Parnuta, G., Tudoroiu, M., Curnel, Y., Thomasset, M., Jensen, V., Knudsen, M. A., Foffová, E., Chandelier, A. and Steenackers, M. 2013. Breeding of broadleaves: Common ash (*Fraxinus excelsior* L.). In *Forest tree breeding in Europe: current state-of-the-art and perspectives*. Pâques, L. E. (ed.). Springer, Vol. 25, pp. 403-462.

Eichhorn, J., Roskams, P., Ferretti, M., Mues, V., Szepesi, A. and Durrant, D. 2010. Visual assessment of Crown Condition and Damaging Agents. 49 pp. Manual Part IV. In: Manual on methods and criteria for harmonized sampling, assessment, monitoring and assessment of the effects of air pollution on forests. UNECE ICP Forests Programme Co-ordinating Centre, Hamburg. ISBN:978-3-926301-03-1. [http://www.icpforests.org/Manual.htm]

Kjaer, ED., McKinney, LV., Nielsen, LR., Hansen, LN. and Hansen, JK. 2012. Adaptive potential of ash (*Fraxinus excelsior*) populations against the novel emerging pathogen *Hymenoscyphus pseudoalbidus*. *Evolutionary Applications* 5: 219-228.

Maes B. (red.) 2006. Inheemse bomen en struiken in Nederland en Vlaanderen: herkenning, verspreiding, geschiedenis en gebruik. Uitgeverij Boom, Amsterdam. 376 pp. (in Dutch)

Mitchell, R.J., Beaton, J.K., Bellamy, P.E., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Gimona, A., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Iason, G.R., Kerr, G., Littlewood, N.A., Newey, S., Potts, J.M., Pozsgai, G., Ray, D., Sim, D.A., Stockan, J.A., Talor, A.F.S. and Woodward, S. 2014. Ash dieback in the UK: A review of the ecological and conservation implications and potential management options. *Biological conservation* 175: 95-109.

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, V. and Bartkevicius, E. 2011. Performance of twenty four European *Fraxinus excelsior* populations in three Lithuanian progeny trials with a special amphasis on resistance to *Chalara fraxinea*. *Baltic Forestry* 17: 17-34.

Roskams, P. and De Haeck, A. 2011. De essenziekte (*Chalara fraxinea*) in het Vlaamse Gewest: een voorlopige stand van zaken. Instituut voor Natuur- en Bosonderzoek, Brussel, INBO.R.2011.49 (in Dutch)

Roskams, P. and De Haeck, A. 2011. Informatieblad essenziekte (*Chalara fraxinea*). Instituut voor Natuur- en Bosonderzoek, Brussel, 4 pp. (in Dutch)

Sioen, G., Verschelde, P. and Roskams, P. 2015. Bosvitaliteitsinventaris 2014. Resultaten uit het bosvitaliteitsmeetnet (Level 1). Instituut voor Natuur- en Bosonderzoek, Brussel, INBO.R.2015.7887244 (in Dutch with English summary)

Waterinckx, M. and Roelandt, B. 2001. De bosinventarisatie van het Vlaamse Gewest. Resultaten van de eerste inventarisatie 1997-1999. Afdeling Bos & Groen, Ministerie van de Vlaamse Gemeenschap, 486 pp. (in Dutch)

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 68 – 78.

Ash dieback in Switzerland: history and scientific achievements

V. QUELOZ[1]*, S. HOPF[2], C. N. SCHOEBEL[3], D. RIGLING[3], A. GROSS[4]

[1] Swiss Federal Research Institute WSL, Swiss Forest Protection, Zuerchestrasse 111, CH-8903 Birmensdorf, Switzerland, [2] Institute for Applied Plant Biology, Sandgrubenstrasse 25/27, CH-4124 Schoenenbuch, Switzerland, [3] Swiss Federal Research Institute WSL, Phytopathology, Zuerchestrasse 111, CH-8903 Birmensdorf, Switzerland, [4] Swiss Federal Institute of Technology ETH, Institute for Integrative Biology, Universitaetsstrasse 16, CH-8092 Zurich, Switzerland
** corresponding author: valentin.queloz@wsl.ch*

Abstract
Monitorings at different spatial scales were done in Switzerland to assess the spread of ash dieback throughout the country. The disease was first observed in 2008 and completely invaded Switzerland seven years later. Most ash trees nowadays suffer to a certain degree from ash dieback. However, the decline process is slow and some trees show tolerance or resistance. Swiss researchers contributed to the current knowledge of ash dieback in different topics: taxonomy and biology of the pathogen, population genetics, host-spectrum as well as biocontrol potential of antagonistic organisms.

Importance of ash in Switzerland

Two species belonging to the genus *Fraxinus* are naturally present in Switzerland: common ash (*Fraxinus excelsior*) and manna ash (*Fraxinus ornus*). Common ash is the second most important broadleaved tree species after beech (*Fagus sylvatica*) in Switzerland. It represents 4.5 % of the stem count (>12 cm DBH) of forest trees distributed over the whole country. On the contrary, manna ash has a very restricted natural distribution range. It is mainly occurring south of the Alps, in the canton Ticino. Young trees of common ash were long considered as weeds by forest practitioners in Switzerland. Indeed common ash has great regeneration abilities and a huge increment growth in the youth, overgrowing other sylviculturally important tree species. Young trees of common ash (<12 cm DBH) account for 21.4 % of the stem count in forest regeneration (Brändli et al. 2014). Common ash is also considered relevant for biodiversity as it provides habitat and niches for numerous organisms such as insects, snails, fungi and lichens (Mitchell 2014). The ecological broad spectrum of ash along with its sylvicultural abilities makes it a very important tree species in Switzerland.

Ash dieback history in Switzerland

Ash dieback monitoring by Swiss Forest Protection

The spread of the disease in Switzerland was monitored by the Swiss Forest Protection (WSL). Surveys on the occurrence of forest pests and diseases are being organized each year with the help of the cantonal forest services. Ash dieback was integrated 2009 in these surveys. First symptoms of ash dieback were recorded 2008 by Roland Engesser (Swiss Forest Protection) in the northwest of Switzerland (cantons Basel and Solothurn) (Engesser et al. 2009). In 2009 the disease was already established in the cantons Jura, Solothurn, Basel and Aargau with only few first signs of disease in canton Zurich. After that, ash dieback progressed continuously towards south, west and east. It appeared 2011 in the inner-alpin areas of the cantons Valais and Graubuenden. South of the Alps, ash dieback was first recorded 2013 near the main mountain range back in the main valley suggesting the pathogen was introduced by wind-dispersed spores from the northern part of Switzerland. By 2015, ash dieback completely invaded Switzerland (Fig. 1a).

Since 2009, distinction was made in the annual surveys between affected young ash trees and crown damage observed on older trees. This is interesting because crown dieback of older ash trees was already reported in Switzerland some years (2004) before ash dieback was officially reported. This phenomenon can be seen in Fig. 1b where ash dieback (on young trees) is restricted to the north of Switzerland while crown damage to older ash trees occurs far more widespread.

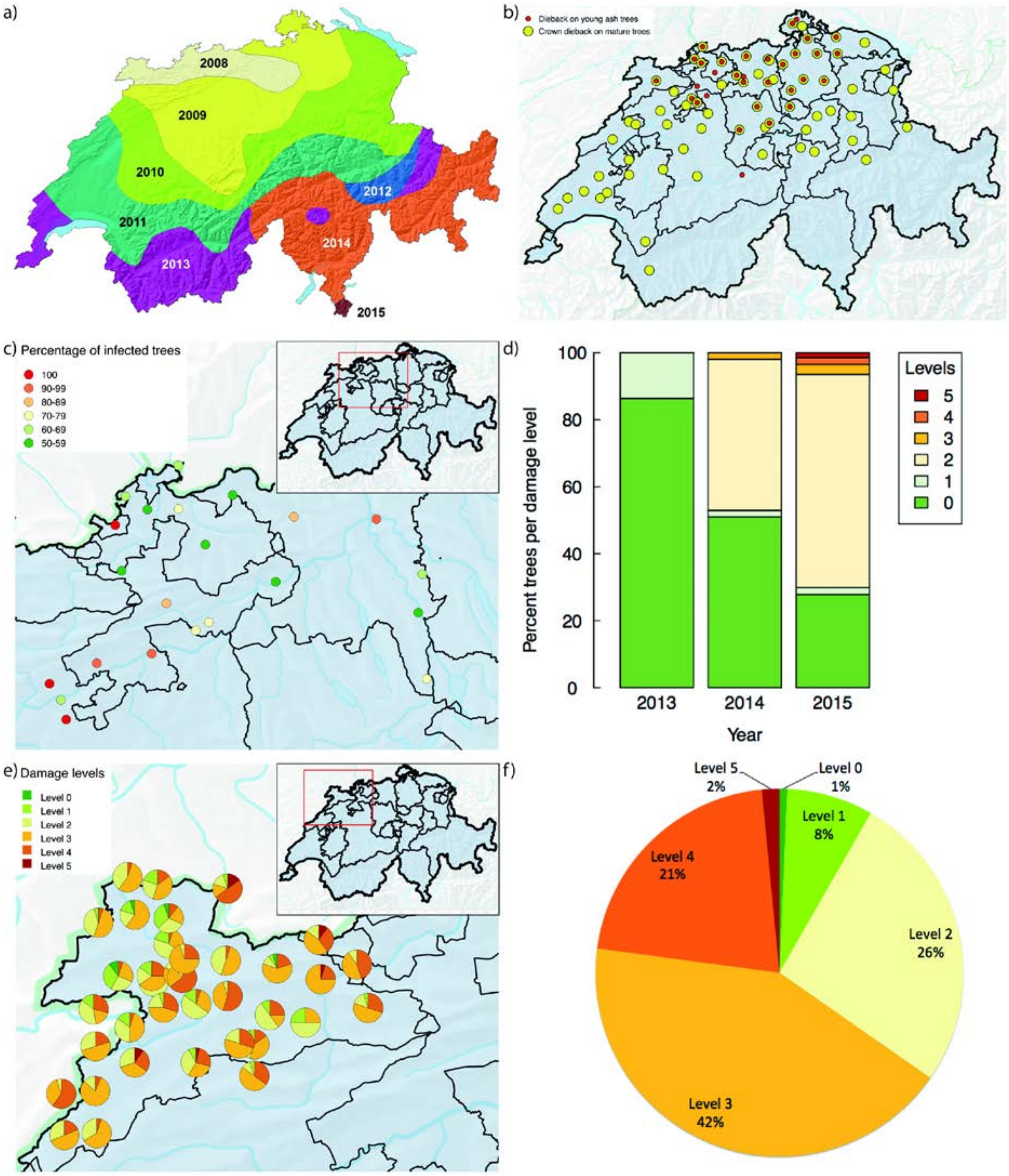


Figure 1a) Ash dieback: invasion history in Switzerland. **1b)** Survey results for 2009. Red dots represent observed damages on young ash trees and yellow dots represent damages of crown on older ash trees. **1c)** Location of the 22 plots in northwestern Switzerland and percentage of affected trees for each observation plot in northwest Switzerland. **1d)** Progress of damages due to ash dieback from 2013 to 2015 in northwest Switzerland (22 observation plots). **1e)** Proportion of the ash trees belonging to the damage levels 0 to 5 within each observed plot in the canton Jura. **1f)** Grouping of the 712 observed ash trees into damage levels 0 to 5 for the canton Jura. geodata © swisstopo.

Local scale monitoring of ash dieback in northwest Switzerland by Institute for Applied Plant Biology

Since 1984, the Institute for Applied Plant Biology (IAP) has been running the Intercantonal Forest Observation Programme on behalf of several Swiss cantons and the Swiss Federal Office for the Environment (BAFU). The focus is on long-term observation of tree health of beech (*Fagus sylvatica*), spruce (*Picea abies*), and oak

(*Quercus* sp.). During the years 2008 to 2015 the presence or absence of ash dieback was monitored by crown assessments in permanent forest observation plots. In 2013, the IAP started a monitoring programme to study the development of ash dieback in Swiss ash stands, with the aim to find some partially resistant trees. By 2015, the programme included 201 trees on 22 plots (Fig. 1c). Because the earliest disease observations were made in 2008 in the northwest of Switzerland, the monitoring program was partly restricted to this region. With the help of local forest administrations, 22 ash stands in northwest Switzerland were chosen for observation. Selection criteria were the following: 1) density of ash trees of ≥50%, 2) DBH ≥10 cm and presence of the disease of about two to three years. In the selected plots between six and ten apparently fully healthy trees were labelled. Every year in July and August, the plots were visually monitored for crown transparency, disease intensity and dead branches (in percentage of total crown volume). Presence of bark discoloration and necrosis were also registered. Subsequently, crown transparency and disease intensity were combined to estimate specific levels of infestation (from level 0 = ‘fully healthy tree’ to level 5 = ‘dead tree’).

After the first year, already 47% of the selected trees showed first symptoms of ash dieback (level 2). In 2014, four new plots with in total 35 still healthy ash trees were included in the monitoring programme. Without these new trees, the portion of affected trees in 2013 would reach 57%. After the second year, the portion of affected trees increased to 70% (141 trees). 128 of these belong to damage level 2 (Fig. 1d). Thus, it will be interesting to observe how long these trees can maintain this low level of infection. Furthermore, six obviously affected (level 3), four heavily affected (level 4) and three dead trees (level 5) were registered. In these cases, the fungus damaged the trees over a very short period of time (one to two years). The proportion of healthy trees (level 0 and 1) during the last assessment in 2015 was 30%. In three plots, all observed trees were affected by the disease (red dots). The lowest percentage of affected trees ranged between 50% and 59%, and was registered in six plots. Most of the plots had an infestation percentage between 60% and 79% (Fig. 1c).

In conclusion, ash dieback is strongly active in all our observation plots. Trees can die within only one or two years. Since 2013, around 50% of the trees without previous symptoms were affected by the fungus every year. On the one hand, there are plots where each tree has been affected; on the other hand, there are still some plots where around 50% of the trees do not show any symptoms yet. There is still good hope to find partially resistant trees in the Swiss forests, i.e. ashes that remain free of any infestation or show minor symptoms over longer time. Therefore, for the local forest service it is important to conserve such trees when thinning and harvesting. In heavily affected stands they will need to look for ecological compensation of ash.

Local scale monitoring of ash dieback in the canton Jura

The canton Jura is located in the northwest of Switzerland. The surface of the canton is 84’000 hectare and 43.5% of its surface is covered with forests. In Jura common ash represents 7% of the stem counts (5% of the total wood volume). Using the canton forest inventory data 2003-2004, 36 plots (0.05 hectares each) were selected containing a minimum of 10 ash trees (>= 20 cm diameter). Two main variables were scored: BHD and tree health ranging from 0 to 5. All plots were visited during June and July 2015. Twenty trees were randomly chosen and measured in each plot. A total of 712 ash trees were recorded in canton Jura.

Most of the trees were considered as diseased and belonged to the damage levels 2 (26%), 3 (42%), and 4 (21%) (Fig. 1f). Surprisingly, less than 2% of the trees were already completely dead (level 5), this is probably due to the ability of ash to create a new crown using epicormic shoots. Around 8% of the ash trees could be considered as relatively healthy, whereas only 5 trees were considered fully healthy (level 0, 1%). An interesting question is the spatial distribution of the disease severity and the differences observed among the measured plots. Records show that some forest plots are on average more affected than others. However, no clear spatial pattern can be detected between health status and geographical location of the plots (see Fig. 1e). Some ecological factors (for example precipitation or geology) may contribute to the observed variation in disease severity.

Some hopeful conclusions can be drawn from the different monitorings of ash dieback in Switzerland. 1) Only very few ash trees are completely dead although most trees are affected by the disease. This gives mature ash trees the chance to reproduce and eventually produce offspring with higher disease tolerance. 2) Around 10% of the ash trees seem to be tolerant / resistant towards ash dieback. This will enable forest practitioners and scientists to develop breeding strategies for the conservation of locally adapted and disease resistant ash trees.

Achievements in ash dieback research

Early research about ash dieback in Switzerland

Research about ash dieback in Switzerland benefited considerably from the Polish forest pathologist Prof. T. Kowalski. Thanks to his friendship and close collaboration with Prof. O. Holdenrieder the disease came into research focus already before the first symptoms arrived in Switzerland. When it became evident that the new disease will have a severe impact on common ash in Europe and the epidemic will soon affect Switzerland the two researchers prepared a first short overview article about the disease for the Swiss Forestry Journal (Kowalski and Holdenrieder 2008). In the same year, the first typical symptoms of ash dieback were reported from northwest of Switzerland (Engesser et al. 2009). At that time, the etiology of the disease was largely unknown. Whereas most forest pathologists were stumbling in the dark, T. Kowalski was on the right track. He repeatedly isolated a putative new hyphomycete fungus of the genus *Chalara* from symptomatic tissues (Kowalski 2001). Later on, he formally described this species as *Chalara fraxinea* while already suggesting its possibly crucial role in the etiology of ash dieback (Kowalski 2006). Subsequently, in collaboration with O. Holdenrieder, Koch's postulates were fulfilled and *C. fraxinea* identified as the main causal agent of the new disease (Kowalski and Holdenrieder 2009a). Soon afterwards, the small discomycete *Hymenoscyphus albidus*, a native decomposer of ash petioles, was identified as teleomorph of *C. fraxinea* (Kowalski and Holdenrieder 2009b). The exciting new findings puzzled researchers because *H. albidus* was known in Europe since the late 19th century and has never attracted the attention of forest pathologists. How could a seemingly harmless, native fungus become suddenly so problematic? This question was addressed in a follow up study of Queloz et al. (2011, 2012). A clever study design allowed the genetic differentiation of a new cryptic species among putative *H. albidus* isolates from diseased and healthy ash stands. The new cryptic species was associated with diseased ash stands and genetically identical to *Chalara fraxinea* isolates. It was named *Hymenoscyphus pseudoalbidus* and later renamed to *H. fraxineus* (Baral et al. 2014) in order to meet the new nomenclature code for fungi (Norvell 2011).

Population genetics of *H. fraxineus* in Europe and Japan

After the identification of the causal agent of ash dieback the logical next question was where the new species came from. This was addressed in the scope of the PhD thesis of A. Gross. Microsatellite markers for the pathogen were established (Gross et al. 2012a, Burokiene et al. 2015, Haňáčková et al. 2015) and populations from different affected regions in Europe were collected. In the meantime, the putative native range of the fungus was identified by other scientists to be East Asia (Zhao et al. 2013). Through collaboration with the authors of this study it was possible to collect different populations of *H. fraxineus* on Manchurian ash *Fraxinus mandshurica* in Japan. Subsequent population genetic analyses of the entire sample collection revealed a severe genetic founder effect in Europe while the bi-allelic nature of microsatellite markers indicated that probably only two individuals were originally introduced in Europe (Gross et al. 2014b). In contrast, the strikingly higher genetic diversity discovered in Japan provided additional support for the hypothesis of an East Asian origin of the fungus. In line with another microsatellite-based population genetic study (Bengtsson et al. 2012), population differentiation within Europe was close to zero, indicating high levels of gene flow among populations. Only a slightly lower genetic diversity was identified at the epidemic front in Switzerland. Two years later, these subtle genetic differences were not detectable anymore. Within the framework of a Swiss-Lithuanian joint research project, Swiss and Lithuanian populations were thoroughly investigated (see below) and again extremely high gene flow levels of the pathogen were detected (Burokiene et al. 2015). Consequently, in the case of *H. fraxineus*, isolation by distance, i.e. the reduction of genetic diversity due to recurrent bottlenecks during spread, is only detectable for a very short time directly at the epidemic front.

Population genetics in Switzerland and Lithuania

In the frame of the Lithuanian-Swiss Cooperation programme a joint research project on ash dieback (CONTROLDIEBACK) between the Lithuanian Nature Research Centre and the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) was initiated. One objective of this research project was to investigate the genetic population structure of the ash dieback pathogen *H. fraxineus* at the epidemic disease front in Switzerland compared to Lithuanian populations, where the disease has been present for a longer time (post-epidemic area). Using microsatellite markers, a total of 847 *H. fraxineus* isolates from five populations in Switzerland and five populations in Lithuania were genotyped (Burokiene et al. 2015). The results of this study showed that there is only little geographic and ecological differentiation of the European *H. fraxineus* populations at the disease front and in the post-epidemic area. All *H. fraxineus* genotypes seem to be able to both induce bark infections on living trees and to colonize leaves, as no genetic differentiation could be detected between isolates recovered from bark lesions and fallen leaf petioles. Notwithstanding the geographic distance between them and the very different dates of the first disease occurrence, the Swiss and Lithuanian populations are genetically very similar. It seems that the entire genetic diversity detected in the founding populations has been transmitted to the epidemic disease front (Switzerland). Nonetheless, gene flow in the Lithuanian population occurs somewhat more random than in the Swiss population and the likelihood of correct assignment of a particular genotype to its population of origin was greater in Switzerland than in Lithuania. This speaks for weak founder effects at the disease front.

Biology of ash dieback

A necessity for a reliable interpretation of population genetic data is the detailed knowledge of the reproduction biology of a species (McDonald 1997). Therefore, the reproduction biology was studied in parallel to the population genetics of *H. fraxineus*. The mating type loci of *H. fraxineus* and *H. albidus* were partially sequenced and described (Gross et al. 2012b, Wey et al. 2016). Interestingly, the two almost indistinguishable species exhibited a different mating type organization – homothallic for *H. albidus* and heterothallic for *H. fraxineus*. A multiplex PCR was developed to quickly distinguish *MAT1-1* and *MAT1-2* isolates. Intra and inter-specific crosses and subsequent microsatellite analyses of progeny isolates revealed or confirmed that (i) *H. fraxineus* is an obligate outbreeder while (ii) frequently more than two genotypes are involved in mating; (iii) *H. albidus* is an obligate inbreeder and (iv) hybridization between the two species is unlikely in nature (could not be forced *in vitro*) (Gross et al. 2012b, Wey et al. 2016). A detailed analysis of the genotype diversity found on ash petioles and subsequent paternal analyses provided evidence that spermatia of the *Chalara* anamorph are involved in fertilization of apothecia. In addition, the analyses showed that petioles are frequently colonized by multiple genotypes whereas matings within petioles only occur between strains with compatible mating types (Gross et al. 2012b). During the study, it became clear that different *H. fraxineus* genotypes are separated on petioles by individual pseudosclerotia – visible as black demarcation- or zone lines when the outer pseudosclerotial plate is tangentially removed. Subsequent experiments showed that the fungus can survive inside petioles under field conditions for two sporulation seasons and withstand drought for at least three months (Gross and Holdenrieder 2013, Gross et al. 2014a). Both findings were later confirmed by Kirisits (2015) whereas he was able to show that the fungus can survive inside petioles under natural conditions for even five sporulation seasons after the leaves have been shed.

The biology of *H. fraxineus* and various aspects of ash dieback were summarized in a review article about the disease (Gross et al. 2014a). In addition, the impact of the disease on *F. excelsior* and its conservation biology consequences were also summarized (Pautasso et al. 2013a, b).

Pathogenicity of *Hymenoscyphus albidus* and *H. fraxineus* on *Fraxinus* spp.

Pathogenicity tests with *H. albidus* and *H. fraxineus* strains from Europe and Japan were conducted on *F. excelsior*, *F. mandshurica* var. *japonica* and *F. pennsylvanica* (Gross and Holdenrieder 2015, Gross and Sieber 2015, Kowalski et al. 2015). *Hymenoscyphus albidus* proved non-pathogenic on all ash species. In contrast, *H. fraxineus* induced necrotic lesions on all *Fraxinus* species tested. This result was surprising especially in the case of *F. mandshurica* which was considered highly resistant/tolerant (Drenkhan and Hanso 2010). Another surprising finding was that *H. fraxineus* strains from Japan caused significantly longer lesions on *F. pennsylvanica* and *F. excelsior* compared to European strains, indicating the potential occurrence of highly virulent strains in Japan (Gross and Sieber 2015).

Searching for a mycovirus

The successful biological control of the chestnut blight fungus *Cryphonectria parasitica* using mycoviruses (Heiniger and Rigling 1994) stimulated the search for mycoviruses in the ash dieback pathogen *H. fraxineus*. Recently, Schoebel et al. (2014) detected a novel mycovirus in European *H. fraxineus* isolates using an RNA sequencing approach. The novel virus could be classified as positive sense ssRNA virus within the genus Mitovirus (family: Narnaviridae). It is called *Hymenoscyphus fraxineus* mitovirus 1 (HfMV1) and is about 2.4 kb in length. HfMV1 is closest related to three mitoviruses of plant pathogenic fungi: *Cryphonectria cubensis* mitovirus, *Sclerotinia sclerotiorum* mitovirus 2 and *Helicobasidium mompa* mitovirus 1-18. Narnaviridae are the simplest of all RNA viruses as they do not have any capsid structure and consist of a single open reading frame, which encodes the viral replicase (RNA dependent RNA polymerase). HfMV1 uses the mitochondrial genetic code suggesting that it is located in the mitochondria of the fungal host. The genus Mitovirus together with the genus Hypovirus (family Hypoviridae) are known to harbour mycoviruses, which induce reduced virulence (hypovirulence) in plant pathogenic fungi and therefore have the potential as biological control agent (e.g. *Cryphonectria hypovirus 1*). The success of a biological control of *H. fraxineus* using mycoviruses will strongly depend on the effective spread of such a virus within *H. fraxineus* populations. The high genotypic and vegetative compatibility (vc) type diversity of the fungal host population (Brasier and Webber 2013, Gross et al. 2014b, Burokiene et al. 2015) may, however, hinder the spread of such viruses that could potentially induce hypovirulence. Unfortunately, up to now a biocontrol potential of HfMV1 could not be confirmed and no further mycoviruses were detected.

Diversity of *Hymenoscyphus* species

During sample collection in Japan, also other ash species were surveyed for the presence of black leaf stalks (an indication of the presence of *H. fraxineus*) in the leaf litter. Petioles of *Fraxinus platypoda* exhibited peculiar dark, narrow, linear pseudosclerotial structures underneath epidermal tissues. Genetic analyses of fungal strains isolated from these pseudosclerotial structures revealed a new *Hymenoscyphus* species closely related to *H. fraxineus*. The formation of the teleomorph could be induced in a moist chamber but subsequent morphological analyses were unable to find morphological differences compared to *H. fraxineus*. Nevertheless, multi-gene phylogenetic analyses clearly separated the fungus from other *Hymenoscyphus* species and the fungus was newly described as *Hymenoscyphus linearis* (Gross et al. 2015). Analyses of herbarium collections of *H. fraxineus* on petioles of *F. mandshurica* and *F. chinensis* from Korea revealed additional cryptic *Hymenoscyphus* diversity. Besides *H. fraxineus*, two other closely related *Hymenoscyphus* species were identified. Using molecular phylogenetics and morphological analyses, these two species were described as *Hymenoscyphus koreanus* and *Hymenoscyphus occultus* (Gross and Han 2015).

Experimental plantation of *F. mandshurica* in Switzerland

An experimental plantation of Japanese proveniences of *F. mandshurica* and *F. platypoda* (syn. *F. spaethiana*) was established 2013 in the Swiss National Arboretum Aubonne (approx. 1400 m^2; canton Vaud; www.arboretum.ch). Before 2013 the arboretum only harbored a small stand of *F. chinensis* ssp. *rhynchophylla* and several single mature trees of various *Fraxinus* species (*F. angustifolia, F. americana, F. excelsior, F. latifolia, F. mandshurica, F. ornus, F. pauciflora, F. pennsylvanica, F. velutina, F. xanthoxyloides;* according to the nomenclature of Wallander (2008).

Symptoms on the planted trees were observed and photographically documented in 2014 and 2015. In addition, *Fraxinus* species were verified by sequencing the ITS region as described in Wallander (2008). On September 3rd 2014, ten *F. mandshurica*, ten *F. platypoda* and seven *F. excelsior* trees were randomly selected and isolations were prepared from two petioles per tree as follows: Petioles were surface sterilized with 99% ethanol using an aerosol can and allowed to dry on a paper tissue. Subsequently, epidermal tissues were removed tangentially using a sterile scalpel. Five adjacent tissue samples, approximately 1 mm^3 in size, were aseptically cut from the inner part of the petiole and immediately transferred to ash leaf malt-extract agar plates (see Gross et al. 2014b). Six samples per species of the same trees (leaflets and remaining petioles) were incubated in moist chambers (petri dish, 15 cm diameter, 3 cm height, containing a moistened paper tissue) at room temperature or in an incubator under near UV-light as described in Gross and Holdenrieder (2013). Moist chambers were checked regularly in order to document the development of leaf symptoms and to detect phialids of the *Chalara* anamorph of *H. fraxineus*. The latter was done using a binocular microscope at 200x enlargement and subsequently verified using a light microscope at 400x enlargement. From two additional *F. mandshurica* trees isolations were carried out directly from symptomatic leaf tissues after 12 days of incubation in a moist chamber using the following method: Leaflets exhibiting necrotic leaf areas were surface sterilized by soaking for 1 min in 70% EtOH, followed by 2 min in 35% H_2O_2 and 1 min in 70% EtOH. Leaflets were dried on a paper towel before eight to nine small pieces of symptomatic tissue pieces, approximately 5 x 5 mm in size, were cut aseptically from the leaflets and immediately transferred to ash leaf malt-extract agar plates (see above). Using these three diagnostic techniques also samples of other *Fraxinus* species were routinely screened for the presence of *H. fraxineus*.

The leaf endophytic fungal microbiome of *F. excelsior, F. ornus* and *F. mandshurica* is currently studied using a metabarcoding approach (PhD Thesis M. Schlegel). The most frequent leaf endophyte in *F. ornus* was described as a new species, *Venturia orni* (Ibrahim et al. 2016).

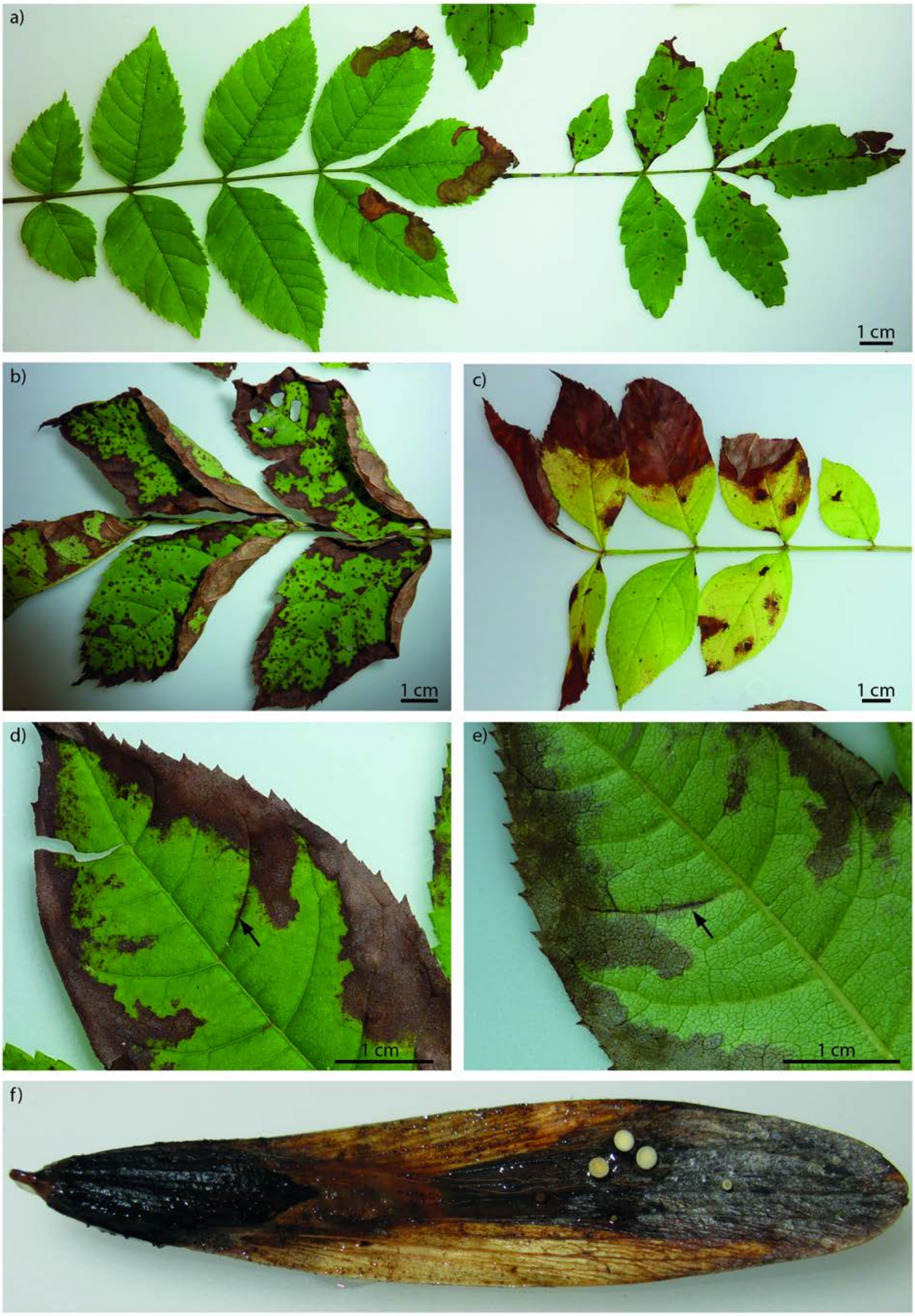


Figure 2 (see next page for descriptions)

Figure 2 Leaf symptoms on *Fraxinus* spp. and fruitbodies of *H. fraxineus* on a samara of *F. xanthoxyloides*. **2a)** Two leaves of the same *F. excelsior* and **2b)** a single leaf of another *F. excelsior* tree showing strikingly different degrees of infection by *H. fraxineus* both within and between tree individuals. **2c)** Leaf symptoms of a *F. mandshurica* tree showing both types of symptoms described in the text. **2d-2e)** Top- (d) and downside (e) of a *F. mandshurica* leaflet showing necrotic leaf areas and one necrosis elongating along the vein. **2f)** Single samara of an *F. xanthoxyloides* tree showing apothecia of *H. fraxineus* after incubation in a moist chamber.

In both years, *F. mandshurica* trees frequently developed leaf symptoms similar to that observed on *F. excelsior* trees (see Fig. 2a-b), including typical necroses elongation along the leaflet veins (Fig. 2c-d-e, 3a-b). At the time of sampling in 2014, shedding of leaves was already in progress but the leaves of some trees were still largely green (e.g. Fig. 3a). Leaves of most trees exhibited necrotic leaf spots. These were either light brown, of irregular size and shape with diffuse border zones towards healthy tissue (Fig. 2c) or dark brown to blackish, regularly round with sharply delimited borders towards healthy tissue (Fig. 3a). In spite of the frequent leaf symptoms, only 4% of *F. mandshurica* trees (n=178) also developed necrotic lesions on shoots until 2015. In two out of ten isolation attempts from shoot lesions, *H. fraxineus* was successfully isolated (Holdenrieder 2015). *Fraxinus platypoda* trees remained largely free of disease symptoms. *Hymenoscyphus fraxineus* was often isolated from petioles of *F. mandshurica* (17/20 isolations positive) and *F. excelsior* (12/14 isolations positive) but never from petioles of *F. platypoda* (20/20 isolations negative). Phialids of the *Chalara*-anamorph were detected frequently on incubated leafs of *F. mandshurica* (5/6 samples positive) and *F. excelsior* (6/6 samples positive) but never on *F. platypoda* (6/6 samples negative). However, on leaves of *F. excelsior*, phialids were much more abundant compared to leaves of *F. mandshurica*, whereas phialids were often restricted to the tissues adjacent to leaflet veins or the petiole itself. Isolation of *H. fraxineus* from symptomatic leaflets was possible in 3 of 5 attempts, of which one example is shown in Fig. 3a-c.

Hymenoscyphus fraxineus could also be detected on the following new hosts: (i) in petioles from the leaf litter and a single twig lesion of *F. pauciflora* (syn. *F. tomentosa*); (ii) in petioles from the leaf litter and from fresh necrotic leaf spots of *F. velutina* and (iii) in petioles from the leaf litter and on seeds of *F. xanthoxyloides* (Fig. 2f).

Single individuals of *Fraxinus* species from the section Melioides (*F. americana, F. latifolia, F. pennsylvanica*) remained largely symptom-free. The pathogen was also not detected in the leaf litter of *F. chinensis* ssp. *rhynchophylla*.

Our analyses suggest that *H. fraxineus* is a frequent leaf blotch pathogen on *F. mandshurica* (planted in Europe) and is capable (to a certain degree) of inducing necrotic lesions on twigs and stems of this species as suggested by Drenkhan and Hanso (2010). This result questions the common believe that *H. fraxineus* occurs endophytically in leaves of *F. mandshurica* in its native range. However, a particular predisposition of *F. mandshurica* due to environmental conditions in Europe is still conceivable.

The finding of three new host species for *H. fraxineus* (*F. pauciflora*, *F. velutina* and *F. xanthoxyloides*) calls for efficient quarantine measures in order to avoid new introductions. Thereby also seeds can serve as a vector, as demonstrated by the fructification of *H. fraxineus* on samaras of *F. xanthoxyloides* (Fig. 2f). This confirms previous findings from genetic analyses of Cleary et al. (2012).

Conclusions and future of ash

Monitorings at different scales helped to assess the intensity and progression of the disease in Switzerland. In 2015, ash dieback reached the whole territory. Although most trees are affected, the decline of mature ash trees takes several years and enables mature trees to reproduce. In addition, few but some common ash trees seem to be tolerant to ash dieback. Defining a countrywide breeding strategy will be the next step for the conservation of common ash in Switzerland. The first test plantings of Asiatic ash species are very promising and research should continue in this direction.

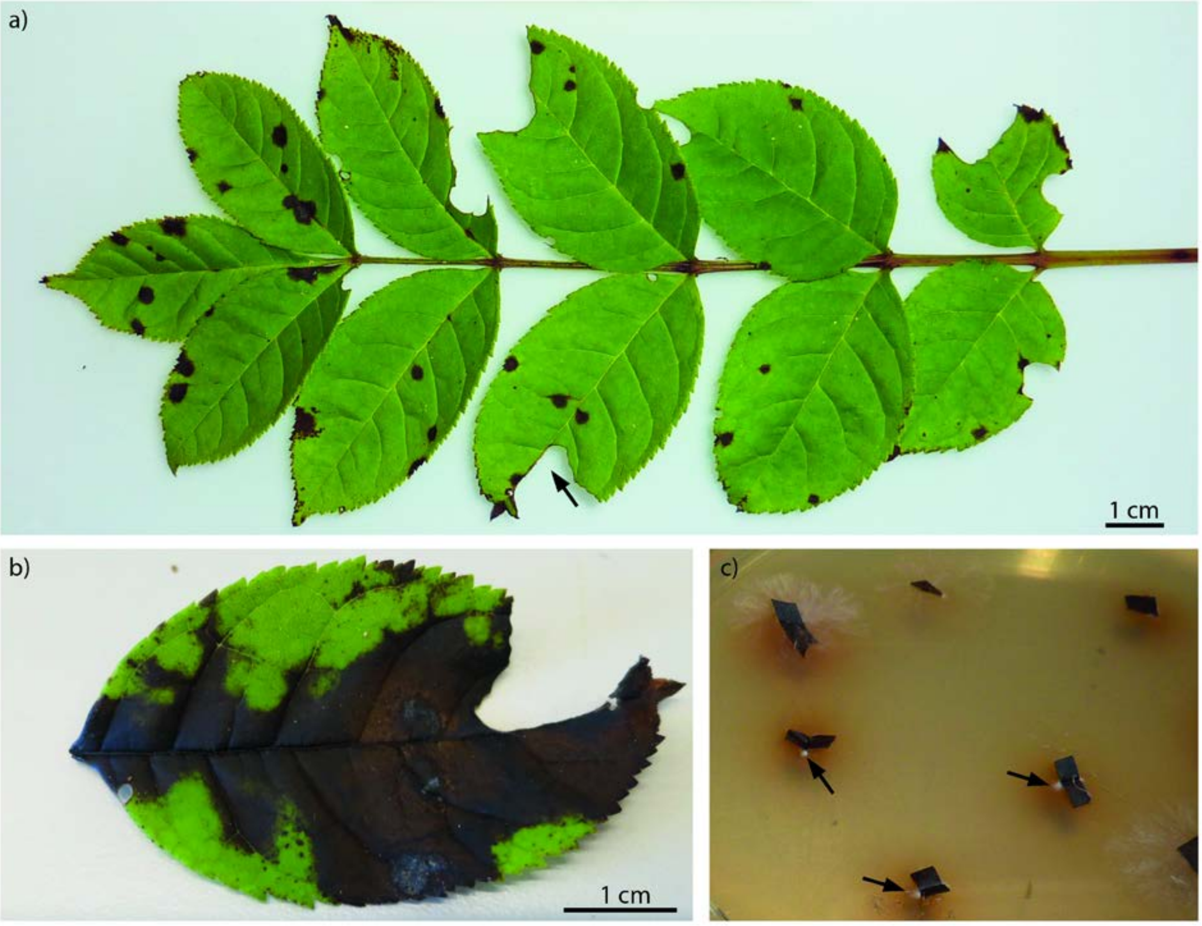


Figure 3 Symptom development in a moist chamber and subsequent isolation of *H. fraxineus*. **3a)** Fresh *F. mandshurica* leaf showing dark brown to black leaf spots. The arrow points to the leaflet which is shown in (3b) and from which *H. fraxineus* was successfully isolated. **3b)** Development of leaf spots after 12 days of incubation in a moist chamber. **3c)** Isolation from necrotic leaf areas of (b). Arrows point to outgrowing mycelia of *H. fraxineus* whereas fast growing mycelia are from other fungi. Also note the characteristic reddish discoloration of the ash leaf malt-extract agar medium, indicating *H. fraxineus*.

Acknowledgements

Many thanks go to H.-O. Baral, J. Han, O. Holdenrieder, T. Hosoya, M. Ibrahim, M. Pautasso, M. Schlegel, T. Sieber, C.R. Gruenig, P. Zaffarano and S. Stroheker from ETH-Zurich, S. Prospero, E. Jung, R. Engesser and F. Meier from WSL, and L. de Witte and S. Braun from IAP for their contribution to the research on ash dieback in Switzerland. We wish to thank the forestry services in Switzerland for taking part in the yearly surveys concerning forest pests and diseases. We would like to thank particularly the 21 local forest administrations supporting the IAP monitoring plots for their good collaboration. Special thanks are for the canton Jura that financed monitoring of ash dieback over his territory. We also acknowledge T. Kowalski for having initiated ash dieback research in Switzerland. Great thanks also go to S. Meier, the National Arboretum of Aubonne and BAFU Project-Nr 2013.01. Finally, we wish to acknowledge COST-Action FRAXBACK FP1103.

References

Baral, H.-O., Queloz, V. and Hosoya, T. 2014. *Hymenoscyphus fraxineus*, the correct scientific name for the fungus causing ash dieback in Europe. *IMA Fungus* 5(1): 79–80.

Bengtsson, S., Vasaitis, R., Kirisits, T. and Solheim, H. 2012. Population structure of *Hymenoscyphus pseudoalbidus* and its genetic relationship to *Hymenoscyphus albidus*. *Fungal Ecology* 5(2): 147–153.

Brasier, C. and Webber, J. 2013. Vegetative incompatibility in the ash dieback pathogen *Hymenoscyphus pseudoalbidus* and its ecological implications. *Fungal Ecology* 6(6): 501–512.

Brändli, U. B., Cioldi, F., Fischer, C., Herold-Bonardi, A., Keller, H.M., Meile, R. et al. 2014. *Viertes Schweizerisches Landesforstinventar - Ergebnistabellen und Karten im Internet zum LFI 2009-2013 (LFI4b)*. Birmensdorf, Eidg. Forschungsanstalt WSL.

Burokiene, D., Prospero, S., Jung, E., Marciulyniene, D., Moosbrugger, K., Norkute, G., Rigling, D., Lygis, V. and Schoebel, C.N. 2015. Genetic population structure of the invasive ash dieback pathogen *Hymenoscyphus fraxineus* in its expanding range. *Biological Invasions* 17(9): 2743–2756.

Cleary, M. R., Arhipova, N., Gaitnieks, T., Stenlid, J. and Vasaitis, R. 2012. Natural infection of *Fraxinus excelsior* seeds by *Chalara fraxinea*. Forest Pathology 43: 83-85.

Drenkhan, R. and Hanso, M. 2010. New host species for *Chalara fraxinea*. *New Disease Reports* 22: 16.

Engesser, R., Queloz, V., Meier, F., Kowalski, T. and Holdenrieder, O. 2009. Das triebsterben der Esche in der Schweiz. *Wald und Holz* 6:24–27.

Gross, A. and Han, J.G. 2015. *Hymenoscyphus fraxineus* and two new *Hymenoscyphus* species identified in Korea. *Mycological Progress* 14(4): 1–13.

Gross, A. and Holdenrieder, O. 2013. On the longevity of *Hymenoscyphus pseudoalbidus* in petioles of *Fraxinus excelsior* Hantula, J. (ed.). *Forest Pathology* 43(2): 168–170.

Gross, A. and Holdenrieder, O. 2015. Pathogenicity of *Hymenoscyphus fraxineus* and *H. albidus* towards *Fraxinus mandshurica* var. japonica. *Forest Pathology* 45(2): 172-174.

Gross, A., Grünig, C. R., Queloz, V. and Holdenrieder, O. 2012a. A molecular toolkit for population genetic investigations of the ash dieback pathogen *Hymenoscyphus pseudoalbidus*. *Forest Pathology* 42(3): 252–264.

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(1): 5–21.

Gross, A., Zaffarano, P. L., Duò, A. and Grünig, C. R. 2012b. Reproductive mode and life cycle of the ash dieback pathogen *Hymenoscyphus pseudoalbidus*. *Fungal Genetics and Biology* 49(12): 977–986.

Gross, A., Hosoya, T. and Queloz, V. 2014b. Population structure of the invasive forest pathogen *Hymenoscyphus pseudoalbidus*. *Molecular Ecology* 23(12): 2943–2960.

Gross, A., Hosoya, T., Zhao, Y. J. and Baral, H.-O. 2015. *Hymenoscyphus linearis* sp. nov: another close relative of the ash dieback pathogen *H. fraxineus*. *Mycological Progress* 14(4): 1–15.

Gross, A. and Sieber, T. N. 2015. Virulence of *Hymenoscyphus albidus* and native and introduced *Hymenoscyphus fraxineus* strains on *Fraxinus excelsior* and *F. pennsylvanica*. *Plant Pathology*: in press.

Haňáčková, Z., Koukol, O., Havrdová, L. and Gross, A. 2015. Local population structure of *Hymenoscyphus fraxineus* surveyed by an enlarged set of microsatellite markers. *Forest Pathology* 45(5): 400–407.

Heiniger, U. and Rigling, D. 1994. Biological control of chestnut blight in Europe. *Annual Review of Phytopathology* 32: 581–599.

Holdenrieder, O. 2015. Ein neuer Ansatz zum Management des Eschentriebsterbens: Anlage einer Versuchspflanzung mit asiatischen *Fraxinus*-Arten. Schlussbericht zum Projekt 2013.01, Fonds zur Förderung der Wald- u. Holzforschung, Bundesamt für Umwelt BAFU, Bern. 37 S.

Ibrahim M., Schlegel M. and Sieber T.N. 2016. *Venturia orni* sp. nov., a species distinct from *Venturia fraxini* living in the leaves of *Fraxinus ornus*. *Mycological Progress*: in press.

Kirisits, T. 2015. Ascocarp formation of *Hymenoscyphus fraxineus* on several-year-old pseudosclerotial leaf rachises of *Fraxinus excelsior. Forest Pathology* 45(3): 254–257.

Kowalski, T. 2006. *Chalara fraxinea* sp nov associated with dieback of ash (*Fraxinus excelsior*) in Poland. *Forest Pathology* 36(4): 264–270.

Kowalski, T. 2001. O zamieraniu jesionów. *Trybuna Leśnika* 4:6–7.

Kowalski T., Bilanski, P. and Holdenrieder O. 2015: Virulence of *Hymenoscyphus albidus* and *H. fraxineus* on *Fraxinus excelsior* and *F. pennsylvanica*. *PLoS ONE* 10(10): e0141592.

Kowalski, T. and Holdenrieder, O. 2008. Eine neue Pilzkrankheit an Esche in Europa | A new fungal disease of ash in Europe. *Schweizerische Zeitschrift für Forstwesen* 159(3): 45–50.

Kowalski, T. and Holdenrieder, O. 2009a. Pathogenicity of *Chalara fraxinea*. *Forest Pathology* 39(1): 1–7.

Kowalski, T. and Holdenrieder, O. 2009b. The teleomorph of *Chalara fraxinea*, the causal agent of ash dieback. *Forest Pathology* 39(5): 304–308.

McDonald, B. 1997. The population genetics of fungi: Tools and techniques. *Phytopathology* 87(4): 448–453.

Mitchell, R. J. 2014. *The potential ecological impact of ash dieback in the UK*. Joint Nature Conservation Committee. 244 p.

Norvell, L. L. 2011. Fungal nomenclature. Melbourne approves a new Code. *Mycotaxon* 116(1): 481–490.

Pautasso, M., Aas, G., Queloz, V. and Holdenrieder, O. 2013a. European ash (*Fraxinus excelsior*) dieback - A conservation biology challenge. *Biological Conservation* 158: 37–49.

Pautasso, M., Aas, G., Queloz, V. and Holdenrieder, O. 2013b. Dieback of European ash: Let's avoid this kind of opportunities for nature conservation . *Biological conservation* 167: 452-453.

Queloz, V., Gruenig, C.R., Berndt, R., Kowalski, T., Sieber, T. N. and Holdenrieder, O. 2011. Cryptic speciation in *Hymenoscyphus albidus*. *Forest Pathology* 41(2): 133–142.

Queloz, V., Grünig, C. R., Berndt, R., Kowalski, T., Sieber, T. N. and Holdenrieder, O. 2012. Corrigendum. *Forest Pathology* 42(4): 352–352.

Schoebel, C. N., Zoller, S. and Rigling, D. 2014. Detection and genetic characterisation of a novel mycovirus in *Hymenoscyphus fraxineus*, the causal agent of ash dieback. *Infection, Genetics and Evolution* 28: 78–86.

Wallander, E. 2008. Systematics of *Fraxinus* (Oleaceae) and evolution of dioecy. *Plant Systematics and Evolution* 273(1): 25–49.

Wey, T., Schlegel, M., Stroheker, S. and Gross, A. 2016. MAT – gene structure and mating behavior of Hymenoscyphus fraxineus and Hymenoscyphus albidus. *Fungal Genetics and Biology* 87: 54–63.

Zhao, Y.-J., Hosoya, T., Baral, H.-O., Hosaka, K. and Kakishima, M. 2013. *Hymenoscyphus pseudoalbidus*, the correct name for *Lambertella albida* reported from Japan. *Mycotaxon* 122(1): 25–41.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 79 – 88.

Ash and ash dieback in the Czech Republic

J. Rozsypálek*, M. Dvořák, R. Longauer, L. Botella, M. Prouza, D. Palovčíková, L. Jankovský

Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 3, 613 00 Brno, Czech Republic
** corresponding author: jiri.rozsypalek@mendelu.cz*

Introduction

At present, forestry often faces the misconducts of previous years. Based on principles of sustainable development forestry has got a far-reaching problem caused by much anthropogenic interference with the natural balance, which alters the structure and species composition of forests. The most important issues include decline and dieback of numerous tree species. Large-scale deforestation followed by forest management requiring production of profitable wood assortments shifted tree species out of its optimal ecological habitats. This approach keeps such forest stands under continuous abiotic stress enhancing their susceptibility to biotic agents. Together with many aspects of global changes trees, forests, plantations and woodlands are endangered by many new incidental factors, often acting in synergies and having significant influence in forest composition and stability of countryside. Extinction or decline of some woody plants has many other consequences, such as dramatic changes in ecosystems, changes in biodiversity due to disappearing of species, lack of traditional raw materials for industry and crafts with a considerable impact on traditions and culture.

In the Czech Republic ash is not a strategic tree species from the point of view of forest and wood production. However, it plays an important role in woodlands and its wood represents a very special material broadly processed by local joineries. Two native species, the common ash (*Fraxinus excelsior*) and narrow-leaved ash (*F. angustifolia*) form riparian vegetation and occupy significant positions in the floodplain forest ecosystem.

The main problems due to Ash dieback (ADB) can be expected in wetter sites with higher occurrence of ash, such as floodplain forests, riparian zones and spring sites (Havrdová et al., 2014). Furthermore, in riparian zones there is a risk of parallel attack of *H. fraxineus* and *Phytophthora alni* on ash and alder, respectively. Further problems can be expected in the ravine forest stands and protective forests on the slopes. The most serious losses occur to young individuals due to lethal course of infection. Also the permanent reduction of elm trees infected by Dutch elm disease produced by *Ophiostoma novo-ulmi* (Dvořák et al., 2007) reduces riparian tree composition. Alders, elms, and common ashes are the main woody plants in riparian forests with an important role stabilizing river banks, however, they are seriously threated by these invasive pathogens.

Among forests ash is an important part of the cultural landscape in windbreaks, avenues and plays an important role in park's plantings. Because of this broad range of ash ecotops we can assume that *H. fraxineus* may cause significant problems. Although it mostly leads to death especially in young plantations (Rozsypálek, 2012), ash dieback spreads without distinction of stand’s age. Natural forests, commercial plantations, forest nurseries, riparian vegetation, alleys, game reserves and urban plantings are attacked.

Importance of ash

Forestry, countryside, urban areas

By 2003 ash (*Fraxinus* spp.) was the sixth most common tree species in the Czech forests covering 1.7% of the forested area (National Forest Inventory, 2006). By 2014, the share of ash in the tree species composition of forests decreased to 1.4% (Ministry of Agriculture of the Czech Republic 2014), which can be attributed to the mortality caused by ash dieback. However, compared to 2004, when the disease was probably already present in the Czech Republic (Jankovský et Holdenrieder, 2009) ash proportion slightly increased. The stock of ash has been gradually increasing from 1990 until 2010 (FAO FRA, 2015) from 4.6 to 6.6 million m^3, what classified it into the group of the top 10 forest tree species from the viewpoint of wood production. Before ADB ash was even mentioned as invasive species in some forest stands and it had to be eliminated from some protected areas.

Distribution of common ash in the Czech Republic ranges from lowlands to mountains. In lowlands it is keystone tree in floodplain forests and on contrary also thermophilic oak stands on limestone slopes and steppes. In highlands ashes are common in alluvia of rivers, creeks and scree slopes, where can even reach mountain areas. The environmental importance of ash mainly consists of fulfilling the water management forest function, stabilizing the banks of streams and transpiration of large amount of water (Šimíček, 1999). In urban areas, such as alleys and parks, ash is the third most common tree followed by maples and limes. The species spectrum of ashes planted is wide but the common ash *(F. excelsior*) considerably prevails.

Tab. 1 Current composition of tree species in the Czech Republic (% of forest land area in the Czech Republic, 2004, 2009, 2014)

Woody plants	Natural composition	2004	2009	2014
Spruce	11.2	53.5	52.15	50.7
Pine	3.4	17.4	16.8	16.5
Fir	19.8	0.9	1.0	1.1
Beech	40.2	6.4	7.2	8.0
Oak	19.4	6.5	6.8	7.1
Larch	0	3.8	3.9	3.9
Birch	0.8	2.9	2.8	2.8
Alder	0.6	1.5	1.6	1.6
Ash	**0.6**	**1.2**	**1.3**	**1.4**
Maple	0.7	1.0	1.2	1.4
Hornbeam	1.6	1.2	1.2	1.3
Lime	0.8	1.0	1.1	1.1
Elm	0.3	0.0	0	0.0
Other	0.6	1.7	1.9	1.9

Source: Ministry of Agriculture of the Czech Republic, Forestry sector reports for years 2004, 2009, 2014.

Tab. 2 Actual composition (2015) of tree species in urban areas in the Czech Republic (%)

Maple	15.8	Pine	4.8
Lime	14.8	Oak	3.8
Ash	**7.2**	Poplar	3.5
Birch	6.8	Horse chestnut	2.5
Prunus varieties	6.6	Crane	2.3
Apple tree	6.0	Acacia	1.5
Spruce	4.8	Other	19.5

Wood industry

More than the half of the ash timber volume is used in the wood processing industry for production of furniture, parquet, veneer, musical instruments, tools and sporting goods, doors, stairs etc. Around 25% of the wood is used for paper production, 7% is used as fuelwood and the rest is used for other assortments of timber production. Use of ash timber in the Czech Republic has very long history, which persists until current times, when traditional materials are highly appreciated for special purposes. The ash wood is for example extraordinary resistant against vibrations because of its elasticity. Therefore, it has been used for skeletons of car bodyworks until early 1950´s (Fig. 1 Aa), handles of tools, sport equipment (Fig. 1 Ac and Ad), short-range bows and arrows have been made from ash timber (for bows with longer range yew and cedar timber are more suitable). It was also used for the manufacture of skids in the sled, or sticks, baseball bats, boats and canoes. Many applications of ash timber are mostly forgotten and replaced by more advanced composites and technologies. The advantage of the unique appearance of ash wood has been taken by folk handicraft (Fig. 1 Ag) and furniture industry (Fig. 1 Ab) - ash timber can be ground and then rubbed into an almost perfectly smooth surface and production of resistant construction elements with exceptional design, such as doors (Fig. 1 Ae), stairs or floors. Other desirable products are ash veneer with a contrasting sapwood and heartwood. Particular properties of ash timber have also been used in the production of musical instruments; especially massive wood of ash, which is a common material in bass guitar bodies (Fig. 1 Af).

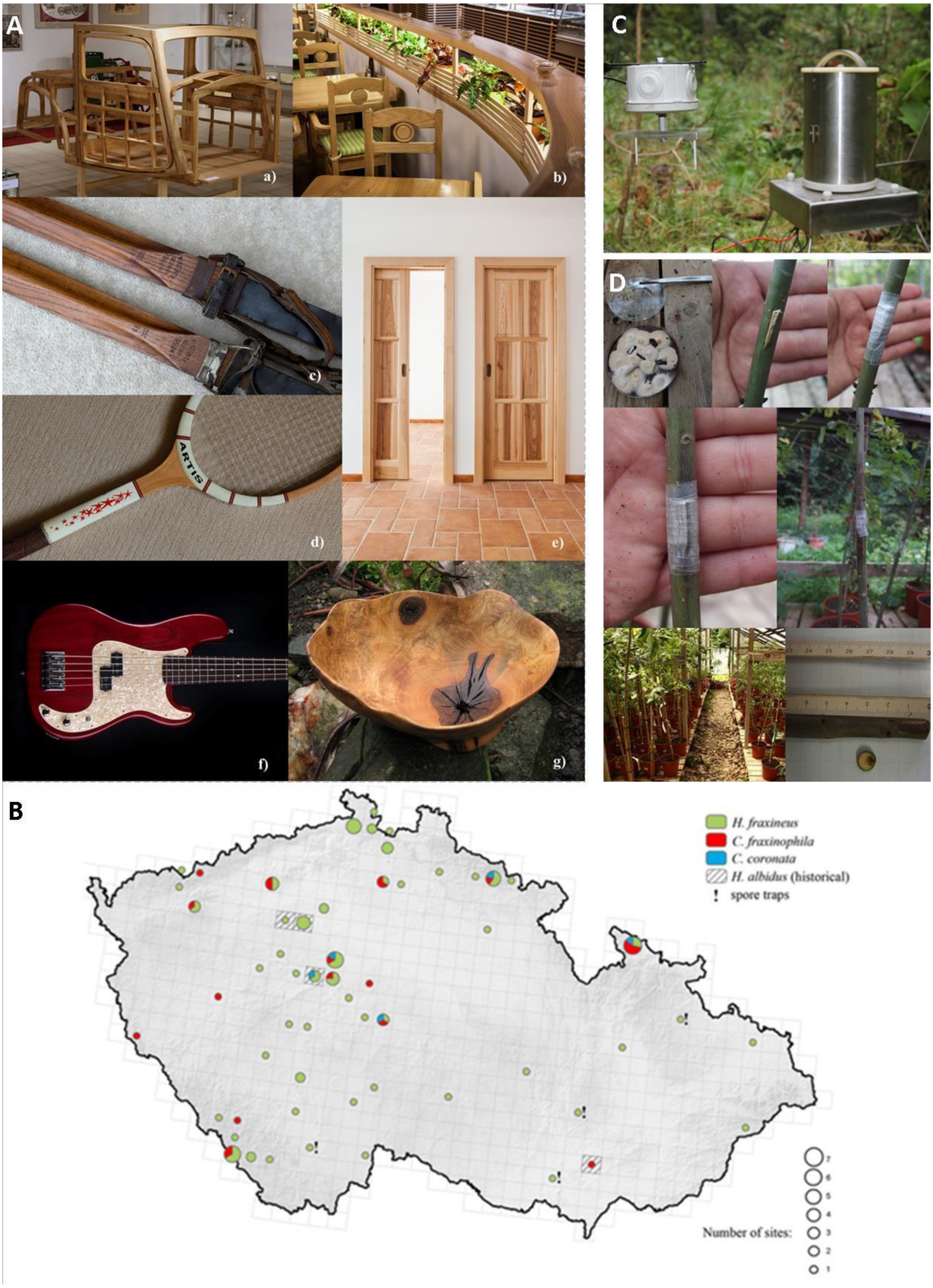


Fig. 1 Aa) Brand new replica (Truhlářství Jiří Hemzal, Vranov, Czechia) of bodywork sceleton for restored old-timer car Z-4 (Zbrojovka Brno, Czechoslovakia) produced in 1930's. Photo M. Dvořák; **Ab)** Furniture made of ash by Truhlářství Bočková (Žďárec, Czechia), Photo H. Bockova, http://www.truhlarstvi-bocek.cz/; **Ac)** Ski made of ash wood by Norgeski,Nossberger (Český Krumlov, Czechoslovakia) in 1930’s. Photo Martin Tůma;

Ad) Tenis racket (Artis Štětí, Czechoslovakia) from 1970's. Photo M. Dvořák; **Ae)** Doors made of ash by Truhlářství Bočková (Ždárec, Czechia), Photo H. Bočková, http://www.truhlarstvi-bocek.cz/; **Af)** Bass guitar with ash body made by Prochazka custom guitars (Prague, Czechia), Photo K. Pazderka, http://www.guitar-makers.com/; **Ag)** Handicraft bowl made of ash (Brno, Czechia), Photo D. Palovčíková. **Fig. 1B)** Map of the *H. fraxineus* occurrence and *H. albidus* historical records detected by Koukol et al., 2016. **Fig. 1C)** Rotating arm (left) and volumetric (right) air samplers employed to sample the inoculum of ADB associated fungi. Photo M. Dvořák. **Fig. 1D)** Procedure of the *H. fraxineus* inoculation. Photo J. Rozsypálek.

Ash dieback and its spread (disease history)

In the Czech Republic symptoms of ash decline locally occur since the late 90s of the last century (there are some herbarium specimens from 2002 from Central Moravia) but since 2004 ash declining has remarkably increased. ADB appeared in Beskydy, Jeseníky, Giant mountains, the Bohemian forest, near Prague, eastern Bohemia, the Czech-Moravian highlands, Drahany highlands, Hostýn in Chřiby (Jankovský et Holdenrieder, 2009). Symptoms of infection (such as drying of crowns, shoot dieback caused by the formation of necrotic spots under the bark) were recorded during the years 2004–2009 with varying intensity throughout the whole area of the Czech Republic (Jankovský et al. 2010).

The confirmation of *Hymenoscyphus fraxineus* in the Czech Republic was firstly reported from samples collected at Drahany highland, arboretum Krtiny, from *Fraxinus excelsior* “Pendula” at the entrance to the arboretum, coordinates: 49°19'7"N/ 16°44'35"E (date of collection: 26th September 2007, sampled by O. Holdenrieder). The symptoms of ADB were already noted in this area in 2004 (Jankovský et Holdenrieder, 2009). Nowadays, *H. fraxineus* is commonly isolated throughout ash stands in the whole Czechia (Fig. 1B; Koukol et al., 2016).

Research regarding ash dieback

The ADB has been investigated thanks to various national research projects founded by the Czech Ministry of Education, Youth and Sports (RDIISCR, 2013), Ministry of Agriculture (RDIISCR, 2012) and by other European projects supported by COST (COST, 2011) and H2020 (EMPHASIS, 2015). Various topics have been developed at universities and research institutes. Below we shortly mention some of the investigated topics and their outputs.

Biology of the pathogen

Environmental factors affecting the epidemiology of *H. fraxineus* have been investigated by Havrdová et Černý (2013). Relationship of the ADB pathogen to the native saprobic species *H. albidus* and others was described by Koukol et al. (2016) and Dvořák et al. (2016), supported by RDIISCR (2012). Haňáčková et al. (2015) dealed with the population structure of *H. fraxineus*. A short work was conducted by Botella et al. (2016) to optimize a medium for isolation and cultivation of the ADB pathogen. Fructification, sporulation and symptoms of ADB in relation to phenology of ashes were studied on several stands in South Moravia within 2013–2015. Significant differences in the phenology, especially flushing time were found between severely and moderately infected trees (Rozsypálek, 2015a).

The spore dispersal pattern and influence of weather was investigated by Dvořák et al. (2016). The authors describe the ascospore dispersal pattern of both *H. fraxineus* and *H. albidus* in one locality in the Czech Republic. The most interesting highlight of this publication is the detected amount of inoculum apart of the expected spore dispersal season with the occurrence of sexual fruiting bodies (apothecia) i. e. April–October. Both species were continuously detected by volumetric and in few cases also by rotating arm air samplers (Fig. 1C). Samples from these instruments were processed by sensitive qPCR method. The possible reasons are discussed, supporting the suggestions of Gross et al. (2012) about the role of conidia in the reproductive process. Results of this work also support the relation between *H. fraxineus* and *H. albidus* inoculum co-occurrence with a clear prevalence of *H. fraxineus* during the whole sampling period, supporting the results of Koukol et al. (2016). Also the determination of inoculum occurrence by weather conditions is investigated. Authors have found significant influence of air humidity and leaf wetness on the inoculum amount with a certain time lag, suggesting the determination of fruiting bodies development and spore release by these factors (Dvořák et al., 2016).

The occurrence of putative, doubled-stranded (ds) viral RNA particles has been investigated in more than 100 Czech isolates (Čermáková, 2014). In total, three different dsRNA bands of *ca* 2.2, 2.5 and 4.5 kb were confirmed in 28.4% of the examined *H. fraxineus* samples (Čermáková, 2014). Statistical analyses have revealed no significant relation between the presence of dsRNA and the growth rate, colour or any other characteristic of the mycelium *H. fraxineus* (Čermáková, 2014). *H. fraxineus* mitovirus 1 (HfMV1) was recorded through high-throughput sequencing of dsRNA in one Czech isolate and confirmed to occur in all isolates presenting bands of *ca* 2.5 and/or 2.2 kb in size using direct specific retro-transcriptase (RT) PCR (Čermáková et al., in preparation). In cooperation with Dr. Daniel Rigling and Dr. Corine Schoebel (Swiss Federal Institute for Forest, Snow and Landscape Research, WSL) the population structure of *H. fraxineus* mitovirus 1 has been studied throughout Europe and Japan (Schoebel et al., in preparation). Furthermore, the presence of HfMV1 has been confirmed in *H. fraxineus* ascospores, which suggests ascospores as the main medium of HfMV1 transmission (Čermáková et Botella, 2015).

Development and impact of ash dieback

Distribution and impact of ADB in forest ecosystems together with the relation of stand conditions on infection intensity was monitored in 300 forest stands using GIS analyses. The ADB severity was assessed using the protocol developed by Rozsypálek (2015a) classifying trees into 10 health condition classes within which trees falling in class 1 and 2 (with lowest infection rate) are considered ADB-tolerant. Trees in class 9 (dead crowns), and 10 (collar and root infections) represent dead trees (Rozsypálek, 2012, 2015a; Prouza, 2015). Results show that severity of impact of ADB is decreasing with the altitude. The most significant differences in the intensity of ADB were noted in floodplain forests around Morava river. In the altitude higher than 760 m a.s.l. trees with lowest impact of ADB were registered (Fig. 2). Only mature pure ash stands were evaluated to avoid the influence of admixed species in early stage of the stand development. However, age of the stand seems to play an important role in the ADB severity of the stand. Young stands up to 50 years are significantly more affected by the ADB than mature stands (Fig. 3), where the trees are damaged with lower intensity (Prouza, 2015).

Symptoms of the ADB were followed weekly during 2013–2015 on experimental plots situated in lowland ash forests in the South-East of the Czech Republic. Heavy impact of the ADB has been recorded since 2008 throughout the whole area. Particular trees showed various dynamic of ADB symptoms development ranging from fast progress of the dieback to trees without any symptom's development. Probably due to extremely hot and dry season 2015 the development of the ADB symptoms was significantly retarded (Rozsypálek, 2015).

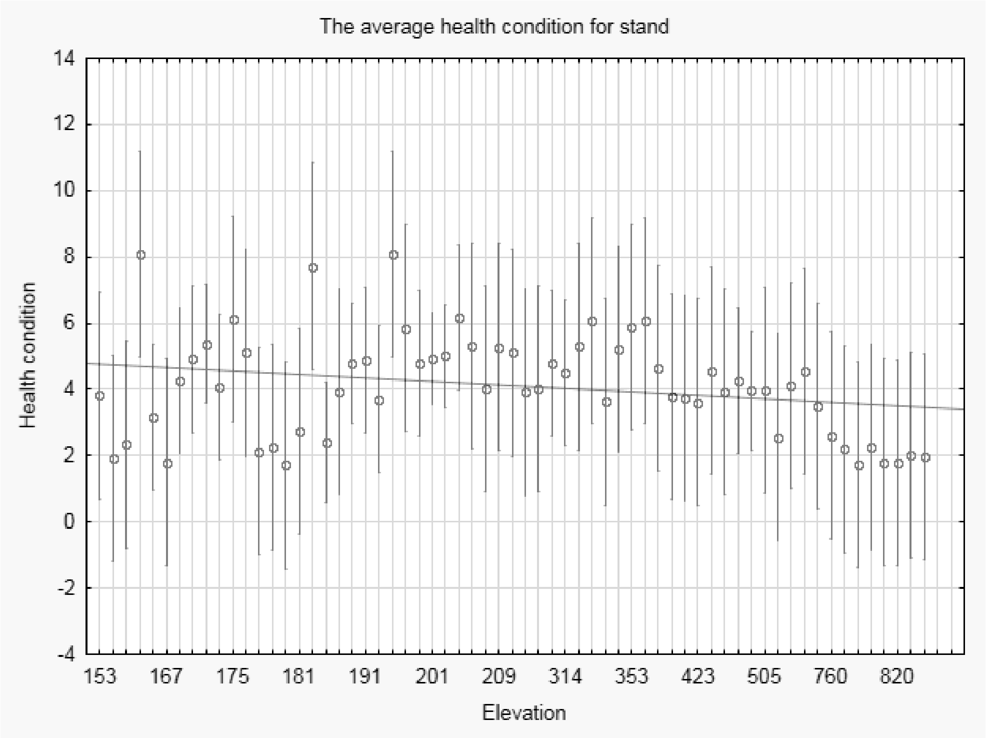


Fig. 2 Health of Ash stands according of altitude (Prouza, 2015).

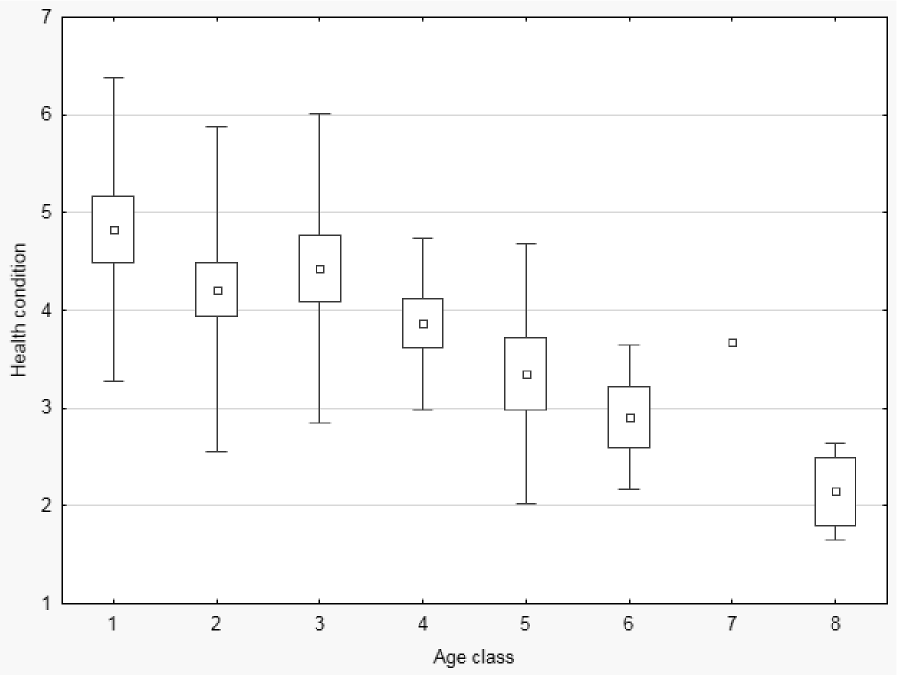


Fig. 3 Classification of health of ash stands according of age classes (1=0–20 years, 2=21–40 years...) (Prouza, 2015).

Genetics of ash populations and resistance

Susceptibility of ashes to *H. fraxineus* infection was studied by inoculation tests with 5 ash species and 4 cultivars. Inoculations were carried out with pure cultures and ascospore solutions of *H. Fraxineus* (Fig. 1D). Preliminary results confirm strong susceptibility of the species *F. excelsior*, especially cv. *'Pendula'* and *'Altena'*, however cv. *'Nana'* was evaluated as the least susceptible cultivar of this species. *F. americana* and *F. ornus* showed the highest tolerance among these experiments (Rozsypálek, 2015b).

Population structure and genetic diversity of ash in Czech Republic

The native Czech ash belongs to the Central European gene pool of species originated in the Alpine glacial refuge according to the phylogenetic studies developed by Heuertz et al. (2004a). The intraspecific variability of different phenotypic traits was studied in different ash provenances of Czech Republic. The experiment including 29 provenances replicated in 5 trial plots in different regions of the Czech Republic, and it was carried out during 1996–1999.

To evaluate the intraspecific variation of ash in relation to the ash dieback, the growth of provenances (heights and DBH of trees), their survival rates and health condition were compared. The health condition of the provenances (= dieback intensity) was assessed using the protocol developed by Rozsypálek (2015a).

Our preliminary results from three replicate plots of the experiment, which are located in eastern part of the Czech Republic (Tab. 3) indicate much better condition and growth of the narrow-leaved ash, which is native in the riverine sites along the Morava river. The two provenances of the species survive as much as twice better than the common ash provenances and their health condition is also significantly better.

Regarding genetic factors, provenance effects proved to be significant in all studied characteristics. They were the biggest in the survival rate and mean diameter of trees, followed by the mean height and health condition (= intensity of ash dieback). The survival rates of the best and poorest common ash provenances differed by 300%, their dieback intensity, phenotypic quality and mean diameter by 40%, and the mean height by 30%. These results are in accordance with Pliura et al. (2011), who reported 2 - to 4 - fold difference in the survival rate, and 50% in the health condition among populations in the geographically broadly based experiment covering Western, Central-European and East-European gene pools of the common ash.

Proportion of potentially resistant ash trees

We estimated proportions of hyposensitive trees of ash also at 5 trial plots of experimental plots established in 1996. The experiment is representative of the ash in the Czech Republic with regard to the number of provenances represented (29) and the fact that seeds of all provenances were collected personally by the founders in the source populations. In addition, there was not silvicultural intervention in 4 plots. Our preliminary results suggest no trend in the current survival rates (Tab. 3). On the other hand, proportion of nearly infection-free "hyposensitive" individuals (coinciding with the "expected final survival") is obviously smaller in the plots located in the western part of the country (Bohemia) which may be with coinciding with climatic differences in the west-east direction.

Tab. 3 Current survival rate and expected final survival rate (based on the proportion of dieback-tolerant individuals) in the series of 5 provenance plots with 30 Czech ash provenances, age 20 years, established by the FGMRI Jíloviště-Strnady.

Trial location	Habitat type	Parent substrate	Altitude	Survival rate - 19 years	Expected final survival*
SE Moravia - Tvrdonice	riparian mixed forest	riverine sediments	155	21,20%	7,20%
Central Moravia - Kroměříž	riparian mixed forest	riverine sediments	190	25,20%	5,50%
NE Bohemia - Deštná	Alder-Ash alluvial forest	sandstone sediments	250	14,80%	1%
Central Bohemia - Koněprusy	Tilio-Acerion slope forest	limestone	350	23,15%	1,80%
East Moravia - Vápenky	Tilio-Acerion slope forest	calcarous flysch	490	21,90%	4%

* Ratio of hyposensitive trees (Class 1 and 2 in the scale of 10) to all originally planted trees in a trial

Management options to mitigate the impact of ash

Silvicultural management options

In young ash, the dieback progresses considerably faster than in adult trees (Rozsypálek, 2015a) because of the size of the host tree but probably also due to higher humidity and higher concentration of spores closer to the ground surface and in understory of forest stands.

However, young stands of the thicket and pole-stage appear to be more threatened by the changed attitude of forest managers towards the species. The indirect effects include direct elimination of the whole groups of declining ash trees including infection-tolerant individuals within them and reduced interest in the silvicultural support to the species by thinning of the species mixtures. Drop in the number of healthy ash seedlings available in the forest nurseries contributes to the overall trend of avoiding ash in the reforestations.

Phenological investigation focused on ashes with different degree of damage by ADB showed clear differences. Severely damaged trees showed a time lag of the spring phenological phases up to one month compared to the healthy trees (Rozsypálek, 2015a). This apparent influence of the pathogen on the seasonal development of the particular trees can indicate targeted silvicultural measures in the spring time. Thus trees with delayed flushing can be removed before further development of the ADB symptoms.

In the both riparian and highland ash sites, potential effects of thinning variants have been tested under the following premises:

- timing (winter and summer thinning);
- thinning method (conventional sanitary thinning aimed to elimination of mean and inferior individuals and positive thinning to support the most vital ash trees).

The hypothesis is that thinning of susceptible individuals in a larger area in winter may have an indirect positive effect thanks to the lower quantity of infected leafs and putatively reduced ascospore discharge in the next vegetation season(s). The effects of thinning will be estimated evaluating the growth, infection intensity and overall health in the thinning variants and the non-intervened plots.

Possible control measures

Due to the rapid spread and high infection pressure of *H. fraxineus* the possibilities of ADB control are limited. In the forest nurseries it is possible to use chemical treatment to protect planted material. But these measures are ineffective, because the plants are protected only when remaining in the nursery. After planting those seedlings into the forest they are heavily damaged and the infection pressure increases in the surrounding vegetation. Chemical control is useless in forest stands. Fungicides used in the large scale can lead to disbalances of macro and micronutrients in the soil and pollution of water sources (Forestry commission, 2005), regardless to the economic impact. The use of fungicides is acceptable only for individual control of valuable trees in countryside and in urban areas.

It seems that the only possibility to preserve ash trees in forests is searching and breeding individuals with increased genetic resistance to *H. fraxineus*. Despite the enormous infection pressure in almost all ash stands there is always a small percentage of minimally affected individuals (Stener, 2012).

In forest ecosystem is appropriate to support those ashes with minimal symptoms and its genetic potential for natural regeneration in stands. Breeding programs with resistant trees are recommended in alleys, river banks, urban areas and artificial plantations. Unfortunately local foresters prefer simple solutions and ash silviculture is dramatically restricted now. Artificial regeneration of ashes is not recommended nowadays because of the high rates of mortality (100%).

So far, biological control does not seem to be a plausible solution to improve the situation of ashes in the Czech Republic. Preliminary investigations carried out to assess a possible effect of fungal viruses on the morphology and growth rate of their fungal hosts did not show statistical significance (Čermáková, 2014). Nevertheless, further experiments should be performed to truly understand the role of mycoviruses within *H. fraxineus*.

Alternative tree species

Severe dieback of trees species, such as alder, elms and ashes has a serious effect on tree diversity. In the softwood floodplain forests genera willows (*Salix* spp.) and poplars (*Populus* spp.) are widely spread but they may suffer from other pathogenic agents as *Dothichiza populea*, *Melampsora* spp., *Xanthomonas populi*, *Agrobacterium* spp. etc. Also native pedunculate oak (*Quercus robur* L.), which remains as the main tree species in the hardwood floodplain forest, shows a significant decline in many localities likely because of climatic and hydrologic reasons. Wild cherry (*Prunus avium* L.) is suitable additional tree species in these stands as well.

Due to decline and dieback of native woody plants in floodplain forest, the importance of other woody plants, including not native species, is increasing. Ash is often replaced by black walnut (*Juglans nigra* L.), but in some habitats there is major risk of introduction of thousand canker diseases caused by *Geosmithia morbida* and transmitted by the walnut twig beetle *Pityophthorus juglandis*. Honey locust (*Gleditsia triacanthos* L.) is used occasionally. The floodplain forest niche vacated by the retreat of ash trees is naturally overgrown with boxelder (*Negundo aceroides* Moe.) and hedge maple (*Acer campestre* L.). However use of non-native trees species is strictly limited by forest and environmental legislation.

The lack of restriction in the use of non-indigenous species in the urban environment is enhancing the ash replacement. To preserve ash in urban environment it is recommended to plant more resistant species and cultivars of ash, such as manna ash (*Fraxinus ornus* L.) or cultivars *F. excelsior* 'Nana', which seems to be more resistant. The use of some cultivars is limited by the absence of suitable resistant rootstock for grafting. Some non-native ashes like *F. pensylvanica* should be also considered (Rozsypálek 2015b).

Conclusions

Ashes in the Czech Republic are until certain point replaceable either from the point of view of wood production, either from the ecological view. To forget ash means to avoid a natural resource, which is historically connected with the culture of the Czech Republic. This traditional material would be missed in many branches of production. Companies and craftsman working with ash timber were never focused on quantity, but on high quality. Design of ash products is mostly highly personalized. Therefore we cannot give up the fight against ADB, support silviculture on the bases of natural regeneration in wide scale and keeping the high genetic diversity.

References

Botella L, Čermáková V, Bačová A, Dvořák M, 2016: ADA, a fast-growth medium for *Hymenoscyphus fraxineus,* Forest pathology 46: 85-87.

Brasier C. M, 1981: Laboratory investigation of Ceratocystis ulmi. In: Compendium of Elm Diseases. Ed. by Stipes R. J, Campana R. J, St. Paul, Minesota, USA: The American Phytopathological Society 76–79.

Čermáková V, Botella L, 2014: Methodology of measurement of mycoviruses transmission within fungal pathogen *Hymenoscyphus fraxineus* spores by using spore traps and qPCR. In *SilvaNet-WoodNet.* 1. vyd. Brno: Mendelova univerzita v Brně, 24-25. ISBN 978-80-7509-137-6, on-line vision, URL:http://www.ldf.mendelu.cz/cz/iga_ldf_mendelu/silvanet__woodnet_2014.

Čermáková V, 2014: Occurrence of putative dsRNA mycoviruses in Ash Dieback Causal Agent. MSc Thesis in Department of Forest Protection and Wildlife Management (FFWT). Mendel University in Brno.

Cornie N. S, Zoller S, Rigling D, 2014: Detection and genetic characterisation of a novel mycovirus in Hymenoscyphus fraxineus, the causal agent of ash dieback. Infection, Genetics and Evolution 28: 78-86.

COST, 2011: European cooperation in science and technology. *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management (FRAXBACK), on-line version, URL:http://www.cost.eu/COST_Actions/fps/FP1103.

Dvořák M, Tomšovský M, Jankovský L, Novotný D, 2007: Contribution to identify the causal agents of Dutch elm disease in the Czech Republic, Plant Protection Science 43: 142-145.

Dvořák M, Rotková G, Botella L, 2016: Detection of Airborne Inoculum of *Hymenoscyphus fraxineus* and *H. albidus* during Seasonal Fluctuations Associated with Absence of Apothecia, Forests 7: 1-13.

EMPHASIS, 2015: Effective management of pests and harmful alien species integrated solutions, on-line version, URL: http://www.emphasisproject.eu/.

Forestry Commision, 2015: Chalara dieback - Managing ash trees and woodland, Citated 25. 4. 2015, Available atWorld Wide Web: http://www.forestry.gov.uk/forestry /INFD-92PJKX#top.

Gross A, Zaffarano P. L, Duo A, Grünig C. R, 2012: Reproductive mode and life cycle of the ash dieback pathogen *Hymenoscyphus pseudoalbidus*, Fungal Genetics Biology, 49: 977-986.

Gross A, et al., 2014: *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. Molecular Plant Pathology. 15: 5-21.

Haňáčková Z, Koukol O, Havrdová L, Gross A, Cleary M, 2015: Local population structure of *Hymenoscyphus fraxineus* surveyed by an enlarged set of microsatellite markers, Forest Pathology 45: 400-407.

Havrdová L, Černý K, 2013: Význam vlhkosti vzduchu v epidemiologii nekrózy jasanu – předběžné výsledky výzkumu (The importance of air humidity in Ash Dieback epidemiology – preliminary results). Zprávy Lesnického Výzkumu 58:347-352.

Havrdová L, Novotná K, Zahradník D, Buriánek V, Pešková V, Šrůtka P, Černý K, 2016: Differences in susceptibility to ash dieback in Czech provenances of *Fraxinus excelsior.* Forest pathology. *In press.*

Koukol O, Haňáčková Z, Dvořák M, Havrdová L, 2016: Unseen, but still present in Czechia: Hymenoscyphus albidus detected by real-time PCR, but not by intensive sampling, Mycological Progres, 15: 1-9.

Jankovský L, Holdenrieder O, 2009: *Chalara fraxinea* – Ash Dieback in the Czech Republic. Plant Protection Science 45:74-78.

Jankovský L, Palovčíková D, 2009: Nový příklad chřadnutí dřevin v ČR – nekróza jasanů (New example of tree decline in Czech Republic), Citated 26. 3. 2015, Available atWorld Wide Web: http://zahradaweb.cz/novy-priklad-chradnuti-drevin-v-cr-nekroza-jasanu/.

Jankovský L, Šťastný P, Palovčíková D, 2009: Nekróza jasanů *Chalara fraxinea* v ČR (Ash Dieback *Chalara fraxinea* in Czech Republic), Lesnická práce 88: 16-17.

Jankovský L, Šťastný P, Palovčíková D, Chlebická M, 2010: Comtemporary situation on Ash dieback in the Czech Republic, EPPO,. Citated 26.3.2015, Available at World Wide Web: http://archives.eppo.int/MEETINGS/2010_conferences/chalara/10_Jankovski/index.html.

Návorovec V, Trejtnarová J, Jančařík V, 2008: Čeká jasany chřadnutí? (Are ash stands expected to decline?), Lesu zdar 5: 4-6.

Prouza M, 2015: Monitoring a ověření metodiky hodnocení nekrózy jasanů způsobené patogenem Chalara fraxinea na vybraných územích (Monitoring and verification of methodology for evaluation ash dieback Chalara fraxinea in selected areas), Bachelor thesis, Mendel university, 109 s.

RDIISCR, 2012: The Research and Development and Innovation Information System of the Czech Republic: QJ1220218 - The development of effective measurements eliminating the impact of *Chalara fraxinea* in forest nursery and in subsequent aspects of forest and water management. (2012-2016, MZE/QJ), on-line version, available at: http://www.isvav.cz/projectDetail.do?rowId=QJ1220218.

RDIISCR, 2013: The Research and Development and Innovation Information System of the Czech Republic: LD13020 - Infection biology of *Chalara fraxinea* and factors influencing fructification of teleomorph *Hymenoscyphus pseudoalbidus*, originator of Ash dieback (2013-2016, MSM/LD), on-line version, available at: https://www.isvav.cz/projectDetail.do?rowId=LD13020.

Rozsypálek J, 2012: Nekróza jasanu *Chalara fraxinea* - fenologie, bionomie, charakteristika symptomy, rozšíření v oblasti Kroměřížska a Zlínska (Ash Necrosis thanks to *Chalara fraxinea* – phenology, bionomy, characteristic of symptoms, expansion in region Kroměříž and Zlín), Bachelor thesis, Mendel university, 103 s.

Rozsypálek J, 2015a: Infekční biologie *Chalara fraxinea* a faktory ovlivňující fruktifikaci teleomorfy *Hymenoscyphus pseudoalbidus* jako zdroje infekce nekrózy jasanu (Infectious biology of *Chalara fraxinea* and factors affecting fructification of teleomorph *Hymenoscyphus pseudoalbidus* as a source of ash dieback infection), Diploma thesis, Mendel university, 107 s.

Rozsypálek J, 2015b: Infection biology of *Hymenoscyphus fraxineus* – inoculation trials on several ash species, grown in urban environment, Nitra, Význam starostlivosti o dreviny vo verejnej zeleni, 31-38.

Stener L, 2012: Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* in southern Sweden, Scandinavian Journal of Forest Research 28: 205-216.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 89 – 105.

Ash dieback in Germany: research on disease development, resistance and management options

R. ENDERLE*[1], B. FUSSI[2], H. D. LENZ [3], G. LANGER[4], R. NAGEL[5], B. METZLER[1]

[1] Department of Forest Health, Forest Research Institute of Baden-Wuerttemberg, Freiburg, Baden-Württemberg, D-79100, Germany, [2] Bavarian Office for Forest Seeding and Planting, Teisendorf, Bavaria, D-83317, Germany, [3] Department of Forest Protection, Bavarian State Institute of Forestry, Freising, Bavaria, D-85354, Germany, [4] Department of Forest Protection, Northwest German Forest Research Station, Goettingen, Lower Saxony, D-37079, Germany, [5] Department of Forest Growth, Northwest German Forest Research Station, Goettingen, Lower Saxony, D-37079, Germany
** corresponding author: rasmus.enderle@forst.bwl.de*

Abstract

Ash dieback caused by *Hymenoscyphus fraxineus* reached Germany at the latest in 2002. Various projects investigating the disease and the genetic resistance in *Fraxinus excelsior* have since then been carried out in the country. This review summarizes these studies, depicting in detail the drastic progression of the disease and its devastating impacts. The potential of the genetic resistance against ash dieback is discussed on the background of the genetic structure of German ash populations. In the end, recommendations for the management of diseased ash populations in Germany are given, which aim to preserve the species, retain genetic resources of ash and propose alternative tree species for typical ash habitats.

Keywords: Ash dieback, *Fraxinus excelsior*, *Hymenoscyphus fraxineus*, Resistance breeding, Forest disease management, Genetic diversity

Introduction

Ash dieback is a severe disease that currently devastates the European populations of *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl. The causal agent is the ascomycete *Hymenoscyphus fraxineus* Baral et al. (anamorph: *Chalara fraxinea* T. Kowalski). Most likely it originates from Far East Asia where it lives non-pathogenically on Asian *Fraxinus* species (Marčiulynienė et al. 2013, Zhao et al. 2013, Baral and Bemmann 2014, Gross et al. 2014b, Zheng and Zhuang 2014). In contrast, it causes necroses on leaves and twigs on the mentioned European species, resulting in massive crown dieback after several years of infection (e.g. Bakys et al. 2009, Kirisits et al. 2009, Skovsgaard et al. 2010, Gross et al. 2014a). Moreover, the fungus is able to colonize the bases of stems (Husson et al. 2012). In combination with other fungi and particularly *Armillaria* spp., this leads to severe basal lesions and collar rots (Lygis et al. 2005, Skovsgaard et al. 2010, Bakys et al. 2011, Husson et al. 2012, Enderle et al. 2013). The disease already led to massive salvage felling and mortality and endangers the future utilization of ash in European forestry. However, high genetic resistance against ash dieback is present in a small fraction of ash individuals, possibly providing a solution to sustain ash species in European forests (e.g. McKinney et al. 2014).

Ash is strongly infested by ash dieback in Germany, where the dramatic consequences of the disease and the lack of knowledge about management options gave rise to a number of research projects. This review summarizes the importance of ash and the spread of ash dieback in Germany. Moreover, studies about the development and impact of the disease and the genetics of ash including genetic resistance in German forests are presented. The review also summarizes management options that are currently recommended in Germany. German research about *Hymenoscyphus fraxineus* and the compounds produced by this fungus (e.g. Junker et al. 2014, Halecker et al. 2014) is not part of this review.

Importance of ash in Germany

In Germany, *Fraxinus excelsior* is the only native ash species and the only ash species with noteworthy relevance in forestry. Moreover, European ash is a popular ornamental tree species in gardens, parks, hedges and avenues. Other ash species such as *F. ornus, F. angustifolia* and *F. pennsilvanica* only play a minor role as ornamental trees in urban areas.

According to the third National Forest Inventory (NFI, data kindly provided by Gerald Kändler), which was conducted in 2012, ash covered an area of about 250,000 ha in Germany, which corresponds to 2.4 % of the total forest area. The ash stock was estimated at 74.7 million solid cubic meters (including bark). Although ash trees can be found all over Germany, its abundance is unevenly distributed. Only on calcareous soils, in flood plain forests and ravine forests ash is a main tree species and thus of special importance in certain regions. About a quarter of the total German ash area (25.3 %) and ash stock (23.8 %) is located in the federal state of Baden-Württemberg in south-western Germany (third NFI).

The ability of natural ash regeneration to dominate and outcompete admixed tree species on certain sites in Germany is called "Vereschung" (Fraxinisation). It has been discussed as a problem in former times, especially because of the high light transmission of ash dominated canopies in mature stands that promotes the emergence of undesired dense layers of grasses and herbaceous plants (e.g. Miegroet 1956, Börth 1990, Wagner 1990, Rysavy and Roloff 1994). In recent decades, however, German forest policies aimed to increase the area of ecologically stable mixed forests (e.g. Mantel 1990, Schriewer 2001, Baumgarten and von Teuffel 2005). In the course of this forest conversion, ash as a native, robust and ecologically and economically valuable tree species had been increasingly planted and its regeneration had been promoted. Moreover, facing climate change, the high drought tolerance of ash had been considered advantageous (Schmidt 2007, Anon 2008). Overall, the area covered by ash in Germany increased by 17.4 % from 2002 to 2012 (second and third NFI). Only in the federal states of Schleswig-Holstein and Mecklenburg-Western Pomerania the area covered by ash slightly decreased during this period of time, which possibly is connected to the relatively early infestation of these states by ash dieback.

The importance of ash in Germany is reflected by a high number of certified seed stands (e.g. 155 stands in Bavaria). Seeds had been harvested regularly until the outbreak of ash dieback. Forestry now runs the risk of losing this economically and ecologically valuable tree species for mixed forests due to the increasingly severe ash dieback.

Disease history of ash dieback in Germany

The first remark in the literature about ash dieback in Germany (Heydeck 2005) reports the incident of typical ash dieback symptoms affecting young *F. excelsior* trees in 2002 in the federal states of Brandenburg, Mecklenburg-Western Pomerania and Saxony-Anhalt (north-eastern Germany). Such widespread distribution in 2002 allows the presumption that the pathogen was already present in Germany some years before. And indeed, already in 2000 / 2001, disease symptoms have been recorded in Mecklenburg-Western Pomerania (pers. com. Paul Heydeck) and Hamburg but not assigned to ash dieback. *Chalara fraxinea* was reported in 2007 for the first time in Germany (Schumacher et al. 2007). Meanwhile, the disease is distributed in all German federal states (Fig. 1). In Schleswig-Holstein, Saxony and Bavaria, disease symptoms were observed in 2005, and *H. fraxineus* was proved to be the causal agent in 2006, 2008 and 2008, respectively. Ash dieback reached Lower Saxony and Baden-Württemberg at the latest in the year 2006, while the pathogen was laboratory-confirmed in 2006 and 2009, respectively. In North Rhine-Westphalia ash dieback was observed in the year 2007 and confirmed in 2008. In Hesse and Thuringia first symptoms and evidence of *H. fraxineus* were reported in 2008 and 2009, respectively. First but unassigned symptoms of ash dieback were recorded in Saarland in the year 2008 and 2009, but the presence of *H. fraxineus* was verified in 2010. The last federal state where ash dieback was observed is Bremen (in the year 2012). In the beginning of the infestation of new areas, the disease and especially disease induced mortality was realised only in young plantations, nurseries or natural regeneration. There was some time lag, until symptoms in adult trees attracted attention (e.g. Schumacher et al. 2007, Metzler 2010). Like on the European level, in Germany no alert or quarantine status could be established during the cryptic dissemination of the disease before it became obsolete. Trade and transportation of ash plant material undoubtedly contributed to the fast spread of the pathogen within Germany.

Development and impact of ash dieback

Ash dieback in provenance trials

In south-western Germany, the development of ash dieback has been monitored in a provenance trial in four different stands consisting of more than 1900 *F. excelsior* trees in total that belong to eight provenances from southern Germany (Metzler et al. 2012, Enderle et al. 2013, Erbacher 2015).

Figure 1 First reports and laboratory-confirmed evidence (bold) of ash dieback caused by *Hymenoscyphus fraxineus* in Germany (●) and percentage of ash in forest areas in German federal states (except city states) according to the German National Forest Inventory 3 (2012): ■ = 0.3 % ash, ■ = 1-1.7 % ash, ■ = 2.1-2.4 % ash, ■ = 3.1-3.6 % ash, ■ = 4.9 % ash. Map created by the Northwest German Forest Research Station in 2016, Dep. Forest Protection B4, compiled by Gitta Langer. Data of the National Forest Inventory 3 (BWI 2012) was provided by Gerald Kändler, FVA-BW. Background source: GeoBasis-DE/BKG2010.

The trial was established in 2005 with three years-old saplings. When data collections started in July 2009, it was possible to record earlier affections of trees retrospectively. Since then they have been repeated every year. Tree individual disease affection was classified according to the number of infected twigs. Results are presented in Figure 2 that shows a massive disease progress. Mortality increased exponentially and reached 28.3 % in 2015. On the other hand, only 1.7 % of the trees in 2015 were symptomless in the crowns. Unfortunately, two of the stands were flooded in July 2014 and data collection was thus carried out in mid-August. During this time, premature leaf fall had already occurred, and the assignment of the trees to the classes 0 – 3 was hampered and thus probably to some part inaccurate.

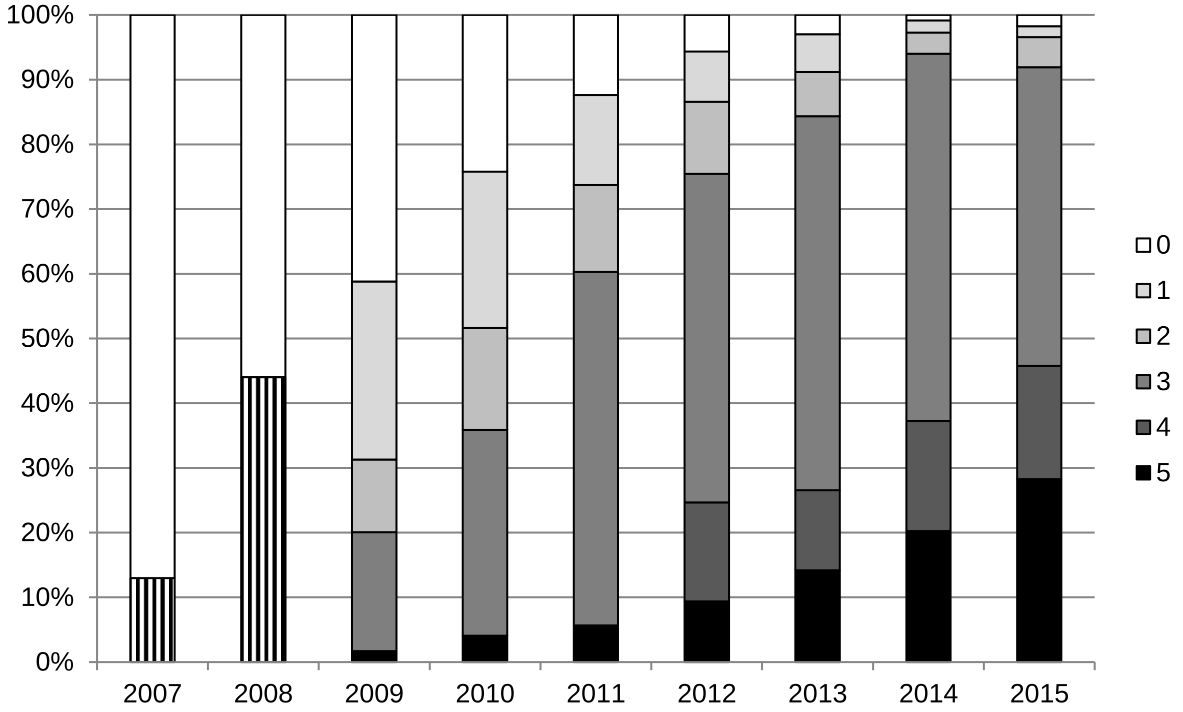


Figure 2 Development of the proportion of trees in classes of disease intensity in a provenance trial from 2007 to 2015, updated from Enderle et al. 2013. For retrospectively analysed years, data are only distinguishing between healthy and affected (hatched bars) individuals. Class 0: healthy, class 1: less than three symptomatic shoots, class 2: less than five symptomatic shoots, class 3: five or more symptomatic shoots, class 4: more than 50 % symptomatic shoots (has been applied since 2012), class 5: dead.

Differences between provenances in crown damage were evident in the beginning of the study (Metzler et al. 2012, Enderle et al. 2013), but became less and less pronounced in the course of disease progress. In 2015, these differences were rather negligible, although still statistically significant (Erbacher 2015).

The presence of collar rots in the trial was recognized in 2011 for the first time and has been recorded systematically since 2012. Molecular investigation of bark samples from collar rots in that year indicated that the involved honey fungus belonged to *Armillaria gallica*. Analyses of the distribution of collar rots in the provenance trial revealed a strong spatial dependency with range from 3.3 m to 11.0 m in different stands. This suggests an influence of micro location conditions on collar rot prevalence. Moreover, collar rot prevalence was highest in the wettest parts of the stands, indicating that collar rot formation is favoured by soil moisture. There was no evidence for differing susceptibility to collar rot between the provenances.

The proportion of living trees with collar rot was 32.6 % in 2012, 46.9 % in 2013, 49.7 % in 2014 and 51.2 % in 2015 (Enderle et al. 2013, Erbacher 2015). Recently, it was shown that susceptibility to collar rot is genetically determined (Muñoz et al. 2015). The little increase of collar rot infections in the last years raises hope that the remaining trees in the trial exhibit relatively high genetic resistance against infections at root collars or are surrounded by micro location conditions that prevent from collar rot formation. By now, collar rot is the most important mortality factor in the provenance trial. Collar rot was present in 92.5 % of the trees that died between 2014 and 2015 and in 84.4 % of the trees that died between 2013 and 2014 (Erbacher 2015).

In a 28 year old provenance trial in Bavaria including fourteen provenances from Germany, two from Switzerland and one from Romania (established in 1988 (Kleinschmit et al. 2002)) ash dieback has been observed since 2011 in every second year. Altogether 592 trees were monitored and damage was recorded following the classification scale elaborated by Lenz et al. (2012). Mortality of the trees increased severely up to 18.6% in 2015, whereas healthy trees dropped down to 3.5% (Figure 3).

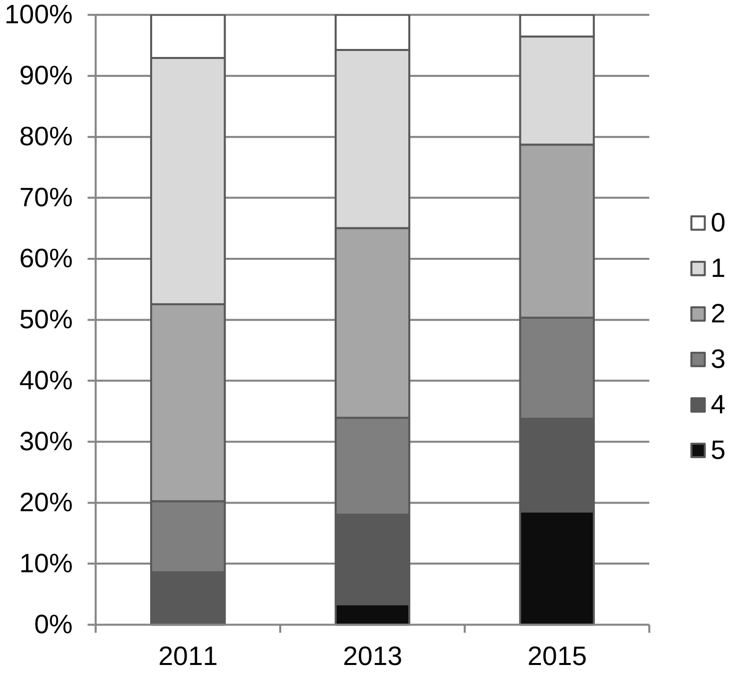


Figure 3 Observation of disease intensity in a provenance trial in Bavaria installed in 1988. Each tree was classified into one of six classes, starting from class 0 for trees with no symptoms to class 5 with 100% damage.

Ash dieback in stands of differing age in southern Germany

In south-eastern Germany, the development of ash dieback, its disease expansion and severity was examined on 22 study sites, capturing differences not only between habitats, but also between age classes (Lenz et al. 2016). The sites included eight mature stands with a total of 230 trees, six pole stands with 584 trees and eight young growth stands with 579 trees. For vitality determination a scoring system was applied that uses six vitality classes (from 0 to 5, Lenz et al. 2012). Depending on their crown defoliation, ash trees belonging to the vitality classes 0 and 1 were less affected, trees within the vitality classes 2 and 3 moderately to seriously affected and trees of the vitality classes 4 and 5 were dying or already dead. Figure 4 shows the development of tree vitality over a period of five years. In 2010, no disease-free areas were found anymore. As we cannot determine how long the fungus has already been present in the different stands and because the initial degree of infestation differed massively (Figure 4), a comparison between the stands is rather difficult. Generally, mortality rates were higher in young growth stands and pole stands compared to mature stands. The development of vitality in some ash stands was not assessed in 2015, because of high mortality rates. With the exception of one young growth stand (No. 24) where only 16 % of the trees were assigned to the vitality classes 4 and 5, all other stands showed mortality rates from more than 50 % up to 95 % in 2014. Three from six pole stands were massively affected by the disease, exhibiting mortality ranging from 37 % to 78 % in 2014. In all mature stands a drastic shift from vitality classes 0 and 1 to 2 and 3 could be observed. Compared to pole and mature stands, the average vitality decreased much faster in most of the young growth stands, even though these stands were already more diseased in the beginning of the monitoring (Lenz et al. 2016). The monitoring will be continued in future. In summary, decreasing vitality accompanied by increasing mortality could be detected in all examined ash stands, regardless of age and habitat. Surprisingly, no correlation between the extent of the mortality and the geographical position of the stands was observed, although the disease was first detected in the southern part of the area of investigation.

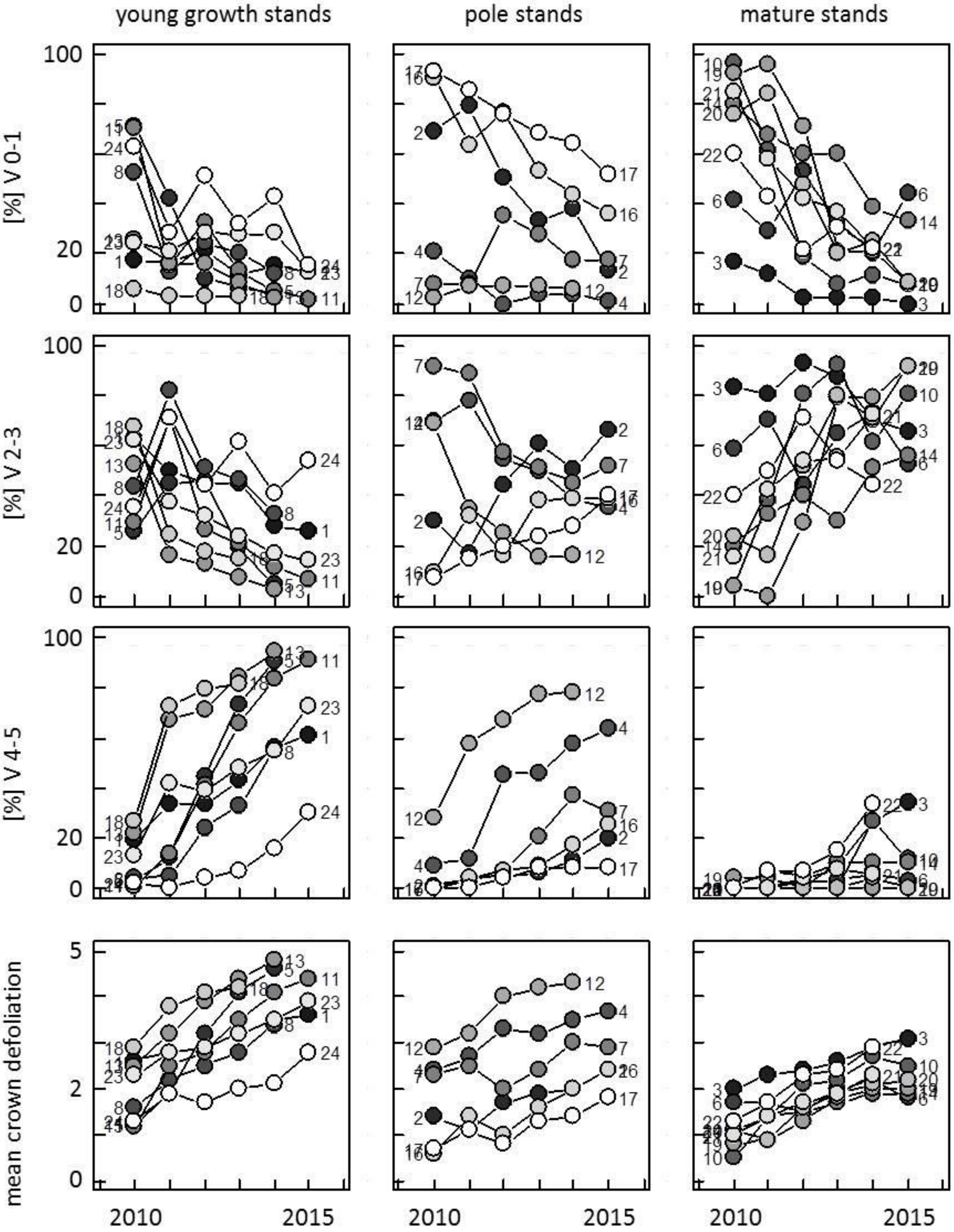


Figure 4 Proportions of trees in classes of crown defoliation V 0-1 (healthy or slightly diseased), V 2-3 (moderately diseased) and V 4-5 (dying or dead) are illustrated for young growth-, pole and mature stands from 2010-2015. The development of the mean crown defoliation (classes 0-5) for each study site is shown at the bottom. Numbers indicate different study sites (updated from Lenz et al. 2016).

Ash dieback in north-western Germany

In north-western Germany, infections and mortality due to ash dieback were monitored in five ash stands of differing age (Langer et al. 2015a). High fractions of infected trees in this and additional ash stands in 2013 and following years demonstrate the fast spread of the pathogen (Table 1). Strong infestations take place in young and old ash stands, natural regenerations and in urban greens, resulting in high mortality. For example, a site afforested with saplings exhibited an infection rate of 80 % one year after planting (Langer et al. 2015a) and 100 % after five years, and mortality added up to 73 %. Especially in Schleswig-Holstein, but also in the other federal states, tree mortality is increasing and often connected with collar necroses. The latter are often associated with *H. fraxineus* or *Armillaria* root rot (Langer et al. 2015b). Therefore, salvage cuttings, especially because of traffic safety responsibilities, had been necessary since 2009 (Langer et al. 2015a). In all investigated sites, the proportion of infected trees and the mortality increased drastically (Table 1). Natural regeneration stands exhibited the highest fraction of healthy trees, but nevertheless were prone to high mortality (Table 1).

Table 1 Mortality and proportions of diseased trees (with regard to crown symptoms) in several ash stands in north-western Germany. na = not applicable because data not compiled.

Location	Forest type	N trees	Tree age 2013	Proportion of diseased trees [%]			Mortality [%]		
Year				2013	2014	2015	2013	2014	2015
Hesse, Neueichenberg	Natural regeneration	543	Ø 5	35	55	80	6	14	38
Hesse, Schotten	Mixed broadleaf forest (natural regeneration)	358	24-125	100	100	100	13	14	22
Lower Saxony, Göttingen	Natural regeneration	489	Ø 3	21	50	71	4	12	23
Lower Saxony, Göttingen	Mixed broadleaf forest (natural regeneration)	318	19-170	100	100	100	0	0,3	1,5
Lower Saxony, Stroit	Afforestation	157	4	99	100	100	62	73	na
Lower Saxony, Stroit	Afforestation	447	15	na	96	99	na	35	39
Lower Saxony, Goseplack	Afforestation	2336	15	100	na	na	36	na	na
Lower Saxony, Riefensbeek	Afforestation	2337	15	100	na	na	7	na	na
Lower Saxony, Elm	Mixed broadleaf forest (natural regeneration)	116	32-134	100	100	100	0,9	3	4
Schleswig-Holstein, Lauenburg	Mixed broadleaf forest (natural regeneration)	220	29-155	100	100	100	15	18	25
Schleswig-Holstein, Scharbeutz	Afforestation	2350	15	100	na	na	54	na	na
Schleswig-Holstein, Satrup	Mixed broadleaf forest	60	93-145	100	100	100	23	23	30

Ash dieback in sample points of the National Forest Inventory

The latest NFI of Germany was conducted in 2012 at permanently marked random sample points in a systematic grid. According to the data of this inventory, ash trees were present at 1.373 sample points of the NFI that are located in the Federal State of Baden-Württemberg in south-western Germany. In July and the first week of August 2015, a special inventory focussing on ash dieback was conducted at a random subsample of 529 of these sample points in order to gain representative data of ash dieback severity in a supra-regional area (Enderle et al. 2015a). This inventory included 1.627 adult ash trees (DBH greater than 7 cm) and 698 regeneration ash trees (taller than 0.5 m and DBH smaller than 7 cm). For every adult tree, crown defoliation and the portion of epicormic shoots of the living crown were assessed in percentage classes. The portion of epicormic shoots turned out to be a suitable indicator for individual susceptibility towards ash dieback (Enderle et al. 2015b). Moreover, the presence or absence of collar rot was recorded for every tree with DBH greater than 7 cm. The data of the regular NFI 2012 was used as a basis to calculate the volume of wood in cubic meters for the respective categories of ash dieback symptoms. For the regeneration trees, ash dieback severity was scored according to the number of infected shoots.

In total, 39.2 % of the total ash stock of Baden-Württemberg, that was 17.8 million cubic meters in 2012 according to the NFI, consisted of ashes with crown defoliation higher than 60 %. Considering these trees as insufficiently vigorous, this means that almost 7 million cubic meters will have to be harvested within the next few years or will decay unused. Ash trees with proportion of epicormic shoots higher than 60 % even accounted for 62.2 % of the total ash stock, giving an indication of the volume of trees that are too susceptible to survive in the long term. Ash trees exhibiting collar rot accounted for 17.5 % of the ash stock. On the other hand, 6.7 % of the ash stock consisted of trees with little crown defoliation and portion of epicormic shoots (less than 25 %) and without collar rot. This fraction of trees might be resistant enough to survive sustainably, although formation of new collar rots is still possible.

The volume of wood consisting of trees that had been harvested or died between the last two inventories (2012 - 2015) was 55.1 % higher for ash than for other tree species on the investigated sample points. Ash trees that died naturally accounted for 10.8 % of this volume, whereas trees of other species that died naturally accounted for only 2.3 % of this volume. This depicts the problem that the timely harvest of the now frequently dying ash trees is not always feasible.

The high sample size allowed the comparison of ecoregions, which revealed considerable differences in the severity of the disease. Crown defoliation and portion of epicormic shoots were smallest in the ecoregion “Neckarland”. Also collar rots were relatively rare in this ecoregion. Forests of the Neckarland are located mainly in colline altitudes of the Keuper Uplands with moderate precipitation. In the ecoregion “Upper Rhine valley”, where ashes exist mainly in flood plain forests, the volume of ashes with collar rots accounted for 42.7 % of the stock, which is the highest proportion of all investigated ecoregions.

The total number of regeneration ash trees decreased drastically by 56.4 % within the three years since the last NFI. The reduction was especially high in the Upper Rhine valley, were ash trees occur mainly in flood plain forests. Here, regeneration trees already weakened by ash dieback are exposed to strong competition by herbaceous plants, particularly when the canopy layer partly disintegrates due to the disease. Of the remaining regeneration trees, 31.9 % were not affected by ash dieback, 14.6 % were diseased at less than 50 % of their twigs, 17.6 % were diseased at more than 50 % of their twigs and 35.8 % of the trees were dead. This proportion of healthy trees is considerably higher than in earlier studies from south-western Germany (Enderle et al. 2016) and is rather comparable to results of a survey in Lithuania (Lygis et al. 2014). However, the vast majority of healthy trees (91.5 %) were smaller than 130 cm in height. Possibly, the high fraction of healthy young ash trees is already a result of commencing evolutionary adaption to the disease.

Genetics of ash populations and resistance

Population structure and genetic diversity of ash in Germany

Several studies used molecular markers to investigate the genetic structure of German ash populations (Höltken et al. 2003, Heuertz et al. 2004a, 2004b, Heblel et al. 2006a, 2006b, Fussi and Konnert 2014). Northern Germany is dominated by one chloroplast haplotype, which is typical for central Europe (Heuertz et al. 2004b). Higher chloroplast variation was detected in southern Germany, especially in populations from south-eastern Bavaria (Fussi and Konnert 2014), where four chloroplast types have been detected by Heuertz et al. (2004b) in the same region. Historical contact of different chloroplast lineages from different refugia in Italy, the eastern Alps and the Balkan Peninsula could explain this pattern (Heuertz et al. 2004b).

The results of genetic studies in Germany revealed a high genetic variation within and high genetic differentiation between ash populations (Hebel et al. 2006b, Fussi and Konnert 2014). High genetic variation within stands was also found in Italy (Ferrazzini et al. 2007) and generally higher genetic variation was detected in central and western Europe than in south-eastern Europe (Heuertz et al. 2004a). In contrast, genetic differentiation was higher for populations in south-eastern Europe than in central Europe (Heuertz et al. 2004a).

Different gene flow intensities during postglacial recolonization might be the explanation for these observed patterns (Heuertz et al. 2004a).

Studies indicate that historical effective seed dispersal occurred mainly over short distances (Heuertz et al. 2004a) and pollen dispersal might have been more effective, since the dispersion of pollen by wind is typical for *F. excelsior*. Nevertheless, the data on pollen dispersal is contradictory, as very limited pollen dispersal has been reported in *F. excelsior* (50% and 95% of pollen dispersed at less than 10 m and 50 m, respectively, Altman and Dittmer (1964) in Heuertz et al. 2001). Douglas (2006) also mentioned that pollen flow and seed dispersal in ash is generally restricted within the stand. These findings could explain the genetic uniqueness of some southern German populations (Fussi and Konnert 2014). However, in some cases gene flow by pollen into fragmented populations can be extensive (46–95%) having two components: a very localized and restricted one and a second one stretching over long distances with dispersal occurring over several kilometres (Bacles et al. 2005). Effective dispersal distances (average 328 m) were greater for fragmented populations than values reported for contiguous populations (Bacles et al. 2005). However, in view of ash dieback, dying trees and / or an active withdrawal of ash can lead to depletion of populations with restricted effective gene flow between the remaining and possibly more resistant trees.

Genetic resistance in populations and individuals

The genetic determinism of the resistance against ash dieback was investigated in four different clonal seed orchards in south-western Germany (Enderle et al. 2015b). In total, the seed orchards comprised 1.726 ramets that belonged to 246 autochthonous clones. The grafts for the clones originated from different nearby regions in south-western Germany. As in former studies, tree individual crown defoliation was assessed in classes in order to score the degree to which the trees were affected by ash dieback. However, some severely diseased trees showed little crown defoliation due to extensive formation of epicormic shoots. Thus, the proportion of epicormic shoots of the living crown was additionally assessed for every tree and interpreted as an indicator for individual susceptibility to ash dieback. The assessments took place in 2012 in all four seed orchards and were repeated in 2013 in two of the orchards. Broad-sense heritability ranged from 0.48 to 0.58 for portion of epicormic shoots and from 0.18 to 0.55 for crown defoliation between the orchards. These results are comparable to other studies from Denmark, Sweden and Lithuania (McKinney et al. 2011, Stener 2013, Pliūra et al. 2014). Differences between the origins of grafts were significant, but very small. Together with the results of the provenance trials, this indicates that the main source of resistance is determined on the level of the individuals, not on the level of populations or provenances.

Fussi and Konnert (2014) reported that ash populations from southern Germany were genetically very different and most of the studied populations displayed symptoms of ash dieback, although in different intensity. Nevertheless, resistance on the population level seems rather unlikely. More likely is that *H. fraxineus* has not arrived in populations, where no symptoms have been discovered yet. In southern Germany, genetic variation between damaged and undamaged subpopulations was compared to see if genetic differences between these two collectives exist (Fussi and Konnert 2014). The authors detected higher proportions of heterozygotic individuals (observed heterozygosity) in the less susceptible groups compared to the susceptible groups, indicating that heterozygotic individuals might be able to withstand ash dieback better than homozygotic individuals. This means a higher variability on the individual level for heterozygotic individuals that might therefor have higher plasticity and reaction capacity against the disease. Namkoong et al. (1998) and Tessier du Cros et al. (1999) earlier suggested that heterozygotic individuals could be more resistant to environmental stresses.

Evolutionary selection in natural ash regeneration

European ash is a tree species, which usually regenerates very successfully. In natural regeneration, densities of more than 100,000 ash trees per ha can be reached (Roloff and Pietzarka 1997, Tabari and Lust 1999), and ash can be very competitive on suitable sites. This fact is worth to consider when searching for management options that aim to increase the resistance against ash dieback in future ash populations. However, ash dieback influences individual competitiveness and changes the processes of evolutionary selection in natural regeneration. To gain a better understanding of selection processes in ash regeneration, individual disease severity was assessed and compared to tree height and annual height growth, which were recorded as indicators of competitiveness (Enderle et al. 2016). The study took place in three sites with dense ash regeneration in south-western Germany with a total number of 1,271 ash trees. Data collections were performed in summer 2013 and winter 2014 / 2015.

In the investigated stands, smaller trees were generally more affected by the disease. The height growth of moderately diseased trees was not reduced compared to completely healthy ashes, but a significant reduction of height growth was evident for trees with more than 50 % crown dieback. Moreover, these trees lost in average 30 % - 40 % of their tree height due to dieback, whereas only minor reduction of height could be detected for less affected trees. The number of living ash trees in the stands decreased by 20.4 % during the period of investigation.

From this study it can be assumed that individual competitiveness is not reduced notably as long as less than half of the twigs of a tree are diseased or dead. However, further research is needed to assess the potential of natural regeneration leading to enhanced resistance by evolutionary selection.

First experiences in breeding for resistance

In order to elucidate the breeding potential and to gain general experience in breeding for resistance against ash dieback, a study was initiated in southern Germany to test the heritability of supposed resistance in the field (Fussi et al. 2016, submitted manuscript). In nine infected mature ash stands, a number of ash trees that visually did not exhibit any disease symptoms were identified in 2010. This can be considered as a first step in a putative breeding program for ash. Scions were collected from these potentially resistant trees and grafted on two years-old root stocks in spring 2012. Following a randomized block design, a field trial was established in Bavaria in winter 2013 / 2014 with 306 of the resulting ramets that belonged to 36 clones. Subsequently, in June 2014 and 2015, individual crown damage was scored by determining the number of infected shoots as a percentage of the total number of shoots per plant.

In 2014, 81.4 % of the ramets and 14 clones were completely healthy. This fraction decreased drastically in the following year, when only 5.6 % of the ramets and no clone were completely healthy. Instead, 11.4 % of the ramets had already died in 2015. There were strong differences in crown damage between the clones but not between the blocks (Kruskal-Wallis tests, $p < 0.001$ and $p = 0.55$, respectively), indicating that the clones differed substantially in their genetic resistance against ash dieback. However, in 2015, only few clones with relatively little crown damage remained. These clones will be further monitored to see if they harbour an adequate potential of resistance for breeding purposes. The relatively high fraction of rather susceptible clones in the trial highlights the importance of thorough determination of the ortets´ degree of resistance. Not more than five years after the pathogen´s arrival in the region (cf. Figure 1) the time of ortet selection (2010) probably was too early. At this time, the amount of pathogen inoculum certainly had not yet been on its maximum, and resistance was not distinguishable from disease absence or disease escape. If possible, ortets for future breeding projects should be selected by thorough observation of the health status over a period of several years under high infection pressure. Younger trees are probably more suitable for the assessment of individual susceptibility, because symptoms can be detected more easily and infection pressure is higher near the ground (Chandelier et al. 2014). Recently identified molecular markers can be used additionally to ensure high degrees of resistance in ash trees that are foreseen for breeding projects (Harper et al. 2016).

Proportion of potentially resistant ash trees

In all examined study sites in Germany, increasing disease progression could be observed that has not come to a standstill yet. As there are currently no efficient treatments known to cure or slow down ash dieback, special attention must be drawn to those trees that withstand the disease for a long period of time under high infection pressure. These trees can be regarded as candidate tolerant trees, although the above described experiences in breeding for resistance reflect the difficulty in assessing a tree´s degree of resistance. Moreover, the identification of candidate trees is much complicated by the symptom of collar rots, which is still not well understood.

According to the studies that are summarized above, the fraction of healthy or slightly diseased trees differs strongly between sites and age classes. In Baden-Württemberg, 6.7 % of the ash stock consists of trees with little crown defoliation and portion of epicormic shoots (less than 25 %) and without collar rots (Enderle et al. 2015a). The current fraction of potential candidate trees in Bavaria has been estimated at 4 % in young plantations and approximately 20 % in mature stands (Lenz et al. 2016). Similar assumptions can be drawn from data that was collected in the provenance trials. However, as the periods of investigations were rather short and because of the huge lack of knowledge concerning collar rots, these estimations are still subject to considerable uncertainty.

However, in natural regeneration that emerged after the infestation, the fraction of healthy trees is much higher (Enderle et al. 2015a, Langer et al. 2015a). This could already be the effect of commencing natural selection towards higher resistance in ash populations.

Ongoing and planned research in Germany

Research regarding ash dieback in southern Germany is foreseen to focus on breeding for resistance and processes of natural selection in autochthonous ash populations, including the genetic component of collar rots. Health stages of ash trees in the provenance trials will be further evaluated. In spring 2016, seedlings produced from seeds of healthy mother trees will be planted in the field. This offspring consists of half-siblings that will provide information on the potential inheritance of resistance of the mother trees and are potential trees for further breeding. But also the pathway of infection leading to collar rots and site characteristics that favour or hamper collar rot formation are intended to be further investigated. A project that compares early infection stages in leaves from susceptible and resistant trees by microscopy has already been initiated in order to gain knowledge about the resistance mechanisms in leaves.

In north-western Germany, it is intended to follow further disease development and its general impact. Mortality and infection rates due to ash dieback as well as the disease progression will be further monitored in at least 12 stands of different age-classes and forest types. The stands are long term observation plots of growth and yield in eastern Schleswig-Holstein, in the eastern and southern highlands of Lower Saxony and the Vogelsberg region in Hesse. The plots already existed before ash dieback appeared. Mixed hardwood stands with in total 1,000 ash trees of different ages ranging from 24 to 157 years are observed. The proportion of ash in the stands ranges from 3 % to 95 %. Disease severity has been assessed annually since 2013 on every numbered ash tree according to a 1 to 5 scoring system (Langer et al. 2015a). It is planned to analyse correlations between tree age, percentage of ash, competition, increment of diameter at breast height and the disease status. The aim of this study is to find conclusions for the maintenance of mixed hardwood stands with ash and to get an idea of the development of stand structure under progressive disease.

Management options to mitigate the impact of ash dieback

Silvicultural management options

Once the devastating effects of ash dieback became obvious in Germany, the forest research institutes of the respective federal states developed independently different recommendations for the management of the disease. Recently, these recommendations to forest practitioners were pooled in a single nationwide publication (Metzler et al. 2013). However, the implementation of the recommended actions is not mandatory in Germany. The following paragraphs give a short summary.

Generally, seed collection, nursing and planting of ash is not recommended. Especially, it is not reasonable to produce healthy but susceptible saplings in nurseries by the application of fungicides. Although detailed data on production of ash plants in Germany do not exist, it is estimated to have dropped down to a 5-10% level in 2015 as compared with the production ten years ago (pers. communication G. Wezel 2016). Existing natural ash regeneration, however, should be utilized in general, although it may be necessary to plant additional site-adapted admixture species if regeneration is dominated by ash. Basically, no further management actions are necessary until the envisaged knot free stem length is reached, but promotion of less competitive admixed tree species and sound looking and resistant appearing ash individuals may be meaningful. Regular thinning in older stands is still strongly recommended. Here again, the promotion of admixed tree species and sound looking ash individuals should be the main objective. Resistant appearing ash trees should never be felled, because it is essential for the conservation of ash to allow these trees to reproduce. Otherwise, fragmentation of ash populations and a crucial loss of genetic diversity must be expected.

Individual tree vigour, as a measure of individual resistance, is the most important criterion during selection of ash trees for thinning. The best time for the selection of trees for harvest is July, when there is a maximum of foliage and disease related crown defoliation can be assessed. Attention must be paid not only to the crown symptoms, but also to collar rots. Permanent marking of final crop ash trees is not feasible anymore. The timber can be used as usual, because it is not a source of additional pathogen inoculum. If massive decline of the canopy and subsequent invasion of grasses or herbaceous plants is to be feared, appropriate measures for forest regeneration must be taken in due time. In mature stands, the main objective should be to harvest high quality timber before deterioration of wood quality, which is increasingly endangered by the formation of epicormic shoots, insects or rot and blue stain fungi when crown defoliation exceeds about 70 %. Trees with collar rots should be harvested in due time, as well.

However, it must be noticed, that foresters are reluctant in selecting potentially resistant ashes and tend to clearcut heavily affected pure stands. This may be due to the incidentally high numbers of collar rots. Within the following years special attention has to be drawn on practical aspects of preserving resistant ash individuals.

During harvest and thinning, high standards for traffic and occupational safety must be applied, which is endangered by dead branches and collar rots (cf. Metzler and Herbstritt 2014). The work should be conducted by qualified staffs that were explicitly informed about the risks. The use of forest harvest machinery is preferable to motor-manual harvesting.

Ash trees in public green, solitary trees in particular, seem to be much less affected by the phenomenon of collar rot, since basal lesions are rarely observed. Thus, the tree safety aspect is much less serious and the responsible staff can concentrate on crown symptoms which can be cured by removing dead branches, possibly to be repeated every year, depending on the progress of disease and on financial background.

Table 2 Alternative tree species to *F. excelsior* based on flooding tolerance and base claim for floodplain and terrestrial forests.

Species	Habitat		Flooding tolerance	Base claim
	Floodplain forest	Terrestrial forest		
Pseudotsuga menziesii[1]		x	low	low
Picea abies[1]	x	x	moderate	low
Larix decidua[1]		x	low	low
Pinus sylvestris[1]	x	x	moderate	low
Abies alba[1]		x	low	middle
Populus tremula[2]	x	x	high	middle
Populus nigra[2]	x		high	high
Salix spp. [2]	x		high	high
Acer pseudoplatanus[1]	x	x	high	high
Ulmus glabra[1]	x	x	moderate	high
Sorbus aucuparia[1]		x	moderate	low
Sorbus torminalis[1]		x	low	high
Acer campestre[1]	x	x	high	high
Ulmus minor[2]	x	x	high	high
Ulmus laevis[2]	x	x	high	middle
Alnus incana[2]	x		high	high
Populus x canescens[2]	x		high	high
Carpinus betulus[1]		x	moderate	middle
Sorbus aria[2]		x	low	high
Betula pubescens[2]	x		high	low
Fagus sylvatica[1]		x	low	low
Betula pendula[1]		x	moderate	low
Alnus glutinosa[1]	x		high	middle
Populus alba[2]	x		high	high
Tilia platphyllos[1]		x	low	high
Sorbus domestica[1]		x	low	high
Acer platanoides[1]	x	x	high	high
Quercus robur[1]	x	x	high	low
Quercus petraea[1]		x	low	low
Prunus padus[2]	x		high	high
Prunus avium[2]		x	low	high
Malus sylvestris[2]	x	x	high	high
Pyrus pyraster[2]	x	x	high	high
Tilia cordata[1]		x	high	middle

[1] According to BaSIS (Taeger et al 2016a, Taeger et al. 2016b)

[2] According to Walentowski et al. 2004

Alternative tree species

In severely affected ash stands, reforestation efforts with alternative tree species have to be considered, especially if mixed species are not available to compensate for the emerging forest gaps. Depending on the habitat (floodplain or terrestrial forest), different species can be used. In south-eastern Germany the Bavarian State Institute of Forestry has developed a digital information system (BaSIS) to describe site conditions (soil and climate) combined with a modeled cultivation risk assessment of tree species (Taeger et al 2016a, Taeger et al. 2016b). By using this system in addition to the appropriate tree species already described in these areas (Walentowski et al. 2004), several alternative tree species could be found for the possible replacement of ash trees (Table 2).

In addition to native species, exotic tree species are considered by some forest owners. However, these are mostly not welcome in close-to-nature forestry and there may be restrictions in protected areas. For example, in flood plain forests, poplar hybrids showed to be able to fill the gaps of declining ash stands. There are also examples of *Juglans nigra* surviving periodic inundation in good health condition. Depending on the forest ownership several experimental plantings were established in order to find tree species that can replace ash adequately.

Conclusions

Generally, ash has relative low abundance in Germany, but forest policies had aimed to increase its share until the devastating effects of ash dieback became apparent. Within ten years, the disease spread over the whole country. Various studies have been carried out in different regions of Germany to follow up the disease progression and to assess the impact of the disease. They revealed a drastic increase in disease severity, which did not come to a standstill, yet. Only a very small fraction of ash trees is still able to withstand the disease. Mortality is increasingly caused by collar rots. The participation of wood decay fungi in collar rots, especially *Armillaria* spp., represents high risks for occupational and traffic safety and thereby is reason for huge salvage cuttings.

It was shown that resistance against ash dieback in Germany is genetically determined on the individual level and that provenances and populations play a negligible role in resistance. In natural populations, genetic diversity was calculated between susceptible and less susceptible trees. The remaining level of genetic diversity within the group of less susceptible individuals has to be monitored, because it is important for breeding programs to ensure a wide genetic base, particularly since analyses based on microsatellites revealed genetic variation in *H. fraxineus* populations (Bengtsson et al. 2012, Fussi et al. 2012, Gherghel et al. 2013) and genotype dependent differences in the pathogen-tree interaction might be expected. The fraction of highly resistant trees that are possibly viable in the long term is still difficult to estimate, especially because the future impact of collar rots is not yet predictable. However, in natural regeneration that emerged after the infestation, natural selection might already have commenced, as the fraction of healthy trees is much higher. Thus, recommendations for disease management in Germany concentrate on the maintenance of sound looking trees to preserve genetic diversity in ash populations.

Acknowledgements

The authors would like to thank the COST-Action FP1103 FRAXBACK that strongly contributed to exchange between ash dieback researchers, international and within Germany, and thereby created an important basis for this review.

References

Anon 2008. Die Esche – zäh und sturmerprobt [Ash – tough and storm-proofed]. *Bayrisches Landwirtschaftliches Wochenblatt* 39: 53-54, (in German)

Bacles, C.F.E., Burczyk, J., Lowe A.J. and Ennos R.A. 2005. Historical and contemporary mating patterns in remnant populations of the forest tree *Fraxinus excelsior* L. *Evolution* 59: 979–990.

Bakys, R., Vasaitis, R., Barklund, P., Ihrmark, K. and Stenlid, J. 2009. Investigations concerning the role of *Chalara fraxinea* in declining *Fraxinus excelsior*. *Plant Pathology* 58(2): 284-292.

Bakys, R., Vasiliauskas, A., Ihrmark, K., Stenlid, J., Menkis, A. and 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.

Baral, H.O. and Bemmann, M. 2014. *Hymenoscyphus fraxineus* vs. *Hymenoscyphus albidus* – A comparative light microscopic study on the causal agent of European ash dieback and related follicolous, stroma-forming species. *Mycology* 5(4): 228-290.

Bartha, B., Mayer, A. and Lenz, H.D. 2016. Acceleration of ash petiole decomposition to reduce *Hymenoscyphus fraxineus* apothecia growth – a feasible method for the deprivation of fungal substrate. *Baltic Forestry* (submitted manuscript)

Baumgarten, M. and von Teuffel, K. 2005. Nachhaltige Waldwirtschaft in Deutschland [Sustainable forest management in Germany]. In: K. von Teuffel, M. Baumgarten, M. Hanewinkel, W. Konold, U.H. Sauter, H. Spiecker and K. Wilpert (Editors), Waldumbau. Springer Berlin, Heidelberg, p. 1-10, (in German)

Bengtson, S.B.K., Vasaitis R., Kirisits, T., Solheim H. and Stenlid J. 2012. Population structure of *Hymenoscyphus pseudoalbidus* and its genetic relationship to *Hymenoscyphus albidus*. *Fungal Ecology* 5: 147–153.

Börth, M. 1990. Vereschung - Problem oder Chance? [Fraxinisation – problem or chance?]. *AFZ-Der Wald* 32: 225-226, (in German)

Chandelier, A., Helson, M., Dvorak, M. and Gischer, F. 2014. Detection and quantification of airborne inoculum of *Hymenoscyphus pseudoalbidus* using real-time PCR assays. *Plant Pathology* 63(6): 1296-1305.

Douglas, G. 2006. Genetic improvement of ash (*Fraxinus excelsior* L.): Abstract of Workshop on Genetics, 18–22 April 2006, Leuven-Belgium, 7 pp.

Enderle, R., Bußkamp, J. and Metzler, B. 2016. Growth performance of dense natural *Fraxinus excelsior* regeneration under attack of the ash dieback agent *Hymenoscyphus fraxineus*. *Baltic Forestry*, (submitted manuscript)

Enderle, R., Kändler, G. and Metzler, B. 2015a. Eschentriebsterben [Ash dieback]. In: Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg (Editor), Waldzustandsbericht 2015 für Baden-Württemberg. FVA, Freiburg, p. 46-54, (in German)

Enderle, R., Nakou, A., Thomas, K. and Metzler, B. 2015b. Susceptibility of autochthonous German *Fraxinus excelsior* clones to *Hymenoscyphus pseudoalbidus* is genetically determined. *Annals of Forest Science* 72(2): 183-193.

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(5-6): 865-876.

Erbacher, J. 2015. Neuste Entwicklung des Eschentriebsterbens an *Fraxinus excelsior* L. in einem Provenienzversuch [Latest development of ash dieback affecting *Fraxinus excelsior* L. in a provenance trial]. Bachelor thesis at the Faculty of Environment and Natural Resources, University of Freiburg, pp 48, (in German)

Ferrazzini, D., Monteleone, I. and Belletti, P. 2007. Genetic variability and divergence among Italian populations of common ash (*Fraxinus excelsior* L.). *Annals of Forest Science* 64: 159–168.

Fussi, B. and Konnert, M. 2014. Genetic analysis of European common ash (*Fraxinus excelsior* L.) populations affected by ash dieback. *Silvae Genetica* 63: 198–212.

Fussi, B., Cremer, E. and Konnert, M. 2012. Neue Möglichkeiten zur Diagnose des Pilzes in Eschengewebe [New possibilities for the diagnosis of the fungus in ash tissues]. *AFZ/Der Wald* 24:13-15, (in German)

Fussi, B., Enderle, R., Karopka, M., Zollner, A., Baier, R. and Konnert, M. 2016. Ansätze zur Resistenzzüchtung gegenüber Eschentriebsterben [Basic approaches to breeding for resistance against ash dieback]. *AFZ-Der Wald*, (submitted manuscript, in German)

Gherghel, F., Fussi, B., Donges, K., Haustein, M., Jakob, K.M., Müller, K., Piškur, B., Hauptman, T., Lenz, H.D., Konnert, M., Kost, G., and Rexer, K.H. 2013. The development of a species-specific test to detect *Hymenoschyphus pseudoalbidus* in ash tissues. *Forest Pathology* 44(2): 137-144.

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.

Halecker, S., Surup, F., Kuhnert, E., Mohr, K.I., Brock, N.L., Dickschat, J.S., Junker, C., Schulz, B. and Stadler, M. 2014. Hymenosetin, a 3-decalinoyltetramic acid antibiotic from cultures of the ash dieback pathogen, *Hymenoscyphus pseudoalbidus. Phytochemistry* 100: 86-91.

Hebel, I., Aldinger, E., Hass, R., Karopka, M., Bogenrieder, A. and Dounavi A. 2006a. Untersuchungen zur Pollenverbreitung und Pollenkontamination in einer Eschen-Samenplantage (*Fraxinus excelsior* L.). *Allgemeine Forst- und Jagdzeitung* 178(2-3): 44–49.

Hebel, I., Hass, R. and Dounavi, A. 2006b. Genetic variation of common ash (*Fraxinus excelsior* L.) populations from provenance regions in southern Germany by using nuclear and chloroplast microsatellites. *Silvae Genetica* 55(1): 38–44.

Heuertz, M., Hausman, J. F., Hardy, O. J., Vendramin, G. G., Frascaria-Lacoste, N. and Vekemans, X. 2004. Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southeastern European populations of the common ash (*Fraxinus excelsior* L). *Evolution* 58(5): 976-988.

Heuertz, M., Hausman, J.F., Tsvetkov, I., Frascaria-Lacoste, N. and Vekemans, X. 2001. Assessment of genetic structure with and among Bulgarian populations of the common ash (*Fraxinus excelsior* L.). *Molecular Ecology* 10: 1615–1623.

Heuertz, M., S. Fineschi and M. Anzidei et al. (2004b): Chloroplast DNA variation and postglacial recolonization of common ash (Fraxinus excelsior L.) in Europe. Molecular Ecology 13: 3437–3452.

Heydeck, P., Bemmann, M. and Kontzog, H.G. 2005. Triebsterben an Gemeiner Esche im nordostdeutschen Tiefland [Shoot dieback on European ash in the north-eastern German lowlands]. *Forst und Holz*, 60(12), 505, (in German)

Höltken, A.M., Tähtinen, J. and Pappinen A. 2003. Effects of discontinuous marginal habitats on the genetic structure of common ash (*Fraxinus excelsior* L.). *Silvae Genetica* 52: 206–212.

Husson, C., Caël, O., Grandjean, J.P., Nageleisen, L.M. and Marçais, B. 2012. Occurrence of *Hymenoscyphus pseudoalbidus* on infected ash logs. *Plant Pathology* 61:889–895.

Junker, C., Mandey, F., Pais, A., Ebel, R. and Schulz, B. 2014. *Hymenoscyphus pseudoalbidus* and *Hymenoscyphus albidus*: viridiol concentration and virulence do not correlate. *Forest Pathology* 44(1): 39-44.

Kirisits, T., Matlakova, M., Mottinger-Kroupa, S., Cech, T. and Halmschlager, E. 2009. The current situation of ash dieback caused by *Chalara fraxinea* in Austria. In: T. Dogmus-Lehtijärvi (Editor) Proceedings of the IUFRO Working Party 7.02.02, Egirdir, Turkey, pp 97-119.

Kleinschmit, J., Lück, F., Rau, H.M. and Ruetz, W. 2002. Ergebnisse eines Eschen-Herkunftsversuchs. [Results from a provenance trial with ash]. *Forst und Holz* 6: 166–172, (in German)

Langer, G. and Werres, S. 2016. Collar Rots in Northwest German forests affected with ash dieback. *Baltic Forestry* (submitted manuscript)

Langer, G., Harriehausen, U. and Bressem, U. 2015a. Eschentriebsterben und Folgeerscheinungen [Ash dieback and its consequences]. *AFZ/Der Wald* 70(20): 22-28, (in German)

Langer, G., Harriehausen, U.,Bressem, U. 2015b. Stammfußnekrosen bei Esche [Collar rots on ash]. *AFZ/Der Wald* 70(20): 29-31 , (in German)

Lenz, H.D., Bartha, B., Straßer, L. and Lemme, H. 2016. Development of ash dieback in south-eastern Germany and increasing occurence of secondary pathogens. *Forests*, (accepted manuscript)

Lenz, H.D., Straßer, L., Baumann, M. and Baier, U. 2012. Boniturschlüssel zur Einstufung der Vitalität von Alteschen [Scoring system for vigour assesments of mature ash trees]. *AFZ-DerWald* 67(3): 18–19, (in German)

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(3): 501-510.

Lygis, V., Vasiliauskas, R. and Stenlid, J. 2005. Wood-inhabiting fungi in stems of *Fraxinus excelsior* in declining ash stands of northern Lithuania, with particular reference to *Armillaria cepistipes. Scandinavian Journal of Forest Research* 20: 337–346.

Mantel, K. 1990. Wald und Forst in der Geschichte - Ein Lehr-und Handbuch [Forests and forestry in history - A textbook and handbook]. Schaper, Hannover, 518 pp, (in German)

Marčiulynienė, D., Cleary, M.R., Shabunin, D., Stenlid, J. and Vasaitis, R. 2013. Detection of *Hymenoscyphus pseudoalbidus* in Primorye region, far east Russia. COST Action FP1103 Fraxback, 4th MC meeting & workshop, 2013 Sep 4–6, Malmö.

McKinney, L.V., Nielsen, L.R., Collinge, D.B., Thomsen, I.M., Hansen, J.K. and Kjær, E.D. (2014). The ash dieback crisis: genetic variation in resistance can prove a long-term solution. *Plant Pathology*, 63(3): 485-499.

McKinney, L.V., Nielsen, L.R., Hansen, J.K. and Kjær, E.D. 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (Oleaceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. *Heredity* 106: 788–797.

Metzler, B. 2009. Eschentriebsterben – *Chalara fraxinea* in Südwestdeutschland [Ash dieback – *Chalara fraxinea* in south-western Germany]. Waldschtz-INFO 4/2009, FVA Baden-Württemberg, Freiburg, 4 pp. (in German)

Metzler, B. 2010. Weitere Entwicklung des Eschentriebsterbens [Further development of ash dieback]. Waldschutz-INFO 3/2010, FVA Baden-Württemberg, Freiburg, 4 pp. (in German)

Metzler, B. and Herbstritt, S. 2014. Sicherheitsrisiko durch Stammfußnekrosen an Eschen, insbesondere auf Nassstandorten [Safety hazards by collar rots affecting ash, particularly on wet sites]. Waldschutz-INFO 1/2014, FVA Baden-Württemberg, Freiburg, 4 pp. (in German)

Metzler, B., Baumann, M., Baier, U., Heydeck, P., Bressem, U. and Lenz, H.D. 2013. Bundesweite Zusammenstellung: Handlungsempfehlungen beim Eschentriebsterben [Nationwide compilation: recommended actions regarding ash dieback]. *AFZ-Der Wald* 68 (5): 17-20, (in German)

Metzler, B., Enderle, R., Karopka, M., Töpfner, K. and Aldinger, E. 2012. Entwicklung des Eschentriebsterbens in einem Herkunftsversuch an verschiedenen Standorten in Süddeutschland [Development of ash dieback in a provenance trial on different south-western German sites]. *German Journal of Forest Research (AFJZ)* 183: 168–180 (in German with English abstract)

Miegroet, M.v. 1956. Untersuchungen über den Einfluss der waldbaulichen Behandlung und der Umweltfaktoren auf den Aufbau und die morphologischen Eigenschaften von Eschendickungen im schweizerischen Mittelland [Investigations on the impact of silvicultural treatment and environmental factors on the structure and the morphological characteristics of ash thickets in the Swiss Plateau]. *Mitteilungen Eidgenössische Anstalt für das forstliche Versuchswesen* 32(6): 229-370, (in German)

Muñoz, F., Marçais, B., Dufour, J. and Dowkiw, A. 2015. Rising out of the ashes: additive genetic variation for susceptibility to *Hymenoscyphus fraxineus* in *Fraxinus excelsior*. doi: http://dx.doi.org/10.1101/031393

Namkoong, G. 1998. Forest Genetics and Conservation in Europe. In: J. Turok, C. Palmberg-Lerche, T. Skroppa and A.S. Ouéraogo (Editors), Conservation of forest genetic resources in Europe, International Plant for Genetic Resources Institute, Rome, pp. 3–10.

Pliūra, A., Marèiulynienë, D., Bakys, R. and Suchockas, V. 2014. Dynamics of genetic resistance to *Hymenoscyphus pseudoalbidus* in juvenile *Fraxinus excelsior* clones. *Baltic Forestry* 20(1): 10-27.

Roloff, A. and Pietzarka, U. 1997 *Fraxinus excelsior* Linné 1753. In: Enzyklopädie der Holzgewächse, Schütt et al. (Editors), 7th edn, ecomed, Landsberg am Lech, pp 1–15. (in German)

Rysavy, T. and Roloff, A. 1994. Ursachen der Vereschung in Mischbeständen und Vorschläge zu ihrer Vermeidung [Causes of fraxinisation in mixed stands and proposals for its prevention]. *Forst und Holz* 49(14): 392-396, (in German)

Schmidt, O. 2007. Vitale Baumart Esche [Vigorous tree species ash]. *LWF aktuell* 58: 20-21, (in German)

Schriewer, K. 2001. Waldbewusstsein und Waldnutzung - eine ökologische Wende [Awareness and utilisation of forests – an ecological change]. *Der deutsche Wald* 51: 24-29, (in German)

Schumacher, J., Wulf, A. and Leonhard, S. 2007. Erster Nachweis von *Chalara fraxinea* T. Kowalski sp. nov. in Deutschland - ein Verursacher neuartiger Schäden an Eschen [First record of *Chalara fraxinea* T. Kowalski sp. nov. in Germany – a new agent of ash decline]. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes*, 59(6): 121-123, (in German)

Skovsgaard, J.P., Thomsen, I.M., Skovgaard, I.M. and Martinussen, T. 2010. Associations among symptoms of dieback in even-aged stands of ash (*Fraxinus excelsior* L.). *Forest Pathology* 40: 7–18.

Stener, L.G. 2013. Clonal differences in susceptibility to the dieback of *Fraxinus excelsior*. *Scandinavian Journal of Forest Research* 28: 1–12.

Straßer, L., Hollersbacher, M., Taeger, S. and Lenz, H.D. 2016. Handlungsempfehlungen für geschädigte Eschenbestände- ein gemeinsames Konzept der Bayerischen Forstverwaltung und den Bayerischen Staatsforsten (BaySF) [Recommendations for damaged ash stands – a common conception of the Bavarian forest administration and the Bayerische Staatsforsten (BaySF)]. *AFZ-DerWald,* (in preperation, in German)

Tabari, K.M. and Lust, N. 1999 Monitoring of natural regeneration in mixed deciduous forest. Silva Gandavensis 65, 58–71.

Taeger, S. and Kölling, C. 2016. Standortinformationssystem BaSIS [Site Information System BaSIS]. *AFZ-DerWald,* (submitted manuscript, in German)

Taeger, S., Jantsch, M. and Kölling, C. 2016. Einfluss besonderer Standortfaktoren auf die Baumartenwahl [Impact of special site effects on tree species selection]. *AFZ-DerWald,* (submitted manuscript, in German)

Tessier du Cros, E., Màtyàs, C. and Kriebel, H. 1999. Contribution of genetics to the sustained management of global forest resources – Conclusions and recommendations. In: C. Màtyàs (Editor), Forest genetics and sustainability, Forestry Sciences, Vol. 63, Kluwer Academic Publishers, Dordrecht, pp. 281–287.

Wagner, S. 1990). Zu: "Vereschung - Problem oder Chance?" [Regarding: ”Fraxinisation – problem or chance?”]. *AFZ-Der Wald* 32: 806-807, (in German)

Zhao, Y.J., Hosoya, T., Baral, H.O., Hosaka, K. and Kakishima, M. 2013. *Hymenoscyphus pseudoalbidus*, the correct name for *Lambertella albida* reported from Japan. *Mycotaxon* 122: 25–41.

Zheng, H.D. and Zhuang, W.Y. 2014. *Hymenoscyphus albidoides* sp. nov. and *H. pseudoalbidus* from China. *Mycological Progress* 13(3): 625-638.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 106 – 114.

Genetics of ash dieback resistance in a restoration context – experiences from Denmark

E. D. KJÆR[1*], L. V. MCKINNEY[1], L. N. HANSEN[1], D. C. OLRIK[2], A. LOBO[1], I. M. THOMSEN[1], J. K. HANSEN[1], L. R. NIELSEN[1]

[1] *Forest Genetics and Diversity; Forest, Nature and Biomass section; Department of Geosciences and Natural Resource Management; Rolighedsvej 23, DK-1958, University of Copenhagen, Denmark,* [2] *Ministry of Environment and Food of Denmark, Nature Agency, Gillelejevej 2B, DK-3230, Denmark,*
** corresponding author: EDK@IGN.KU.DK*

Abstract
Based on experiences from Denmark, we discuss how deployment of natural genetic resistance can support restoration of common ash forests while maintaining their genetic diversity and patterns of local adaptation. We describe how knowledge from genetic studies were obtained and implemented in planning and initiation of on-going activities with large scale screening, testing and deployment of healthy trees as seed sources for future planting of ash in the Danish forests. We discuss the long term perspectives for restoring the decimated ash forests through wise genetic management.

The history of ash dieback in Denmark

The first symptoms of ash dieback were reported in 2003 when Danish foresters observed unusual damage of branches on young ash trees. Soon the problem was reported across the country, but the cause of this severe problem remained unknown. The geographic spread of symptoms from the first observations in Poland in the early 1990's to subsequent observations of similar symptoms in Latvia, Estonia, Belarus, Sweden, Germany, Slovakia, Denmark, and later towards more western and southern countries pointed towards a new emerging infectious disease. However, not until 2006 it was confirmed that the symptoms were caused by the fungus *Chalara fraxinea* (Kowalski & Holdenrieder, 2009; Bakys et al. 2009). The taxonomy and infection biology of the pathogen was not resolved until some years later (Queloz *et al* 2011; Gross et al. 2012). At that time the devastating effects on the ash forests could be observed throughout Denmark, and the logging of ash trees had been accelerated to rescue timber quality. Planting of new ash forests had almost come to a complete halt. The former important forest species had begun to gradually disappear from the Danish forests.

Evidence of genetic variation in resistance

The first Danish pilot study of genetic variation in susceptibility among ash trees was made in 2007 using existing clonal seed orchards present in the country. Historically, ash was the first tree species to be included in breeding activities in Denmark. The Danish Tree Improvement Station (today, Nature Agency) established the first clonal seed orchard (BIRKEMARKEN) in 1947 in cooperation with the Hørsholm Arboretum (today, The University of Copenhagen). This seed orchard was based on 9 clones from plus trees selected at various locations in Denmark due to their superior stem form (Nielsen et al 2009). A few years later, two additional clonal seed orchards (GURRE VANG and TISVILDE) were planted, but like BIRKEMARKEN also based on only a few clones. In 1998, the Danish ash program was diversified by planting two new clonal seed orchards (TUSE NÆS and TAPSØRE) based on 39 clones (McKinney et al 2011). To further diversify, two seedling seed orchards (RANDERS and SILKEBORG) were established in 2004 based on offspring from 101 seed trees selected across the landscape in the western part of Denmark (Kjær et al 2012). Also, a field trial (HØRSHOLM) intended for the study of inbreeding depression was established in 1999, and this trial included single tree offspring from clones in the old Danish clonal seed orchards (Lobo et al. 2015) as well as offspring from 43 randomly selected, mature forest trees from a few forest sites (Lobo et al. 2014). The presence of these five Danish trials was fortunate, because the pedigreed trees made it possible to initiate a rapid survey of genetic variation in susceptibility several years before the disease etiology was clarified.

The first approach was to assess the health of the 39 clones replicated in two clonal seed orchards in 2007 to test if there was genetic variation among the clones in their level of symptoms. A high number of replicates in the clonal seed orchards allowed a relative precise estimation of differences among clones, and made it possible to study whether the same pattern was present at the two sites. The overall mortality and damage level was high, but the results in term of genetic variation was encouraging: the ramets (grafts) of a few clones remained healthy although scattered among heavily infected trees (Olrik et al. 2007, Fig. 1A) in both the TUSE NÆS and TAPSØRE seed orchards. Similar observations were made simultaneously in a parallel study in Sweden (Stener 2013), and it was thus clear at an early stage of the epidemic that genetic variation was present among clones in their levels of susceptibility to the new disease.

The findings from the clonal seed orchards suggested that adaptive potential towards the new disease is likely to be present. In 2007 the origin and spread of the serious disease was still unresolved and ash dieback had only been reported in Denmark for a few years. At least three important questions therefore remained to be addressed before drawing conclusions on the potential role of genetic diversity in fighting the new disease:

- Were the observed differences among clones only reflecting slower development of the symptoms, or would some clones continuously be protected against development of the disease?
- Did low levels of susceptibility reflect a temporary disease escape from abiotic or biotic stress factors, or did the results reflect an active defense mechanism present in some genotypes of ash? And was the level of susceptibility correlated to other important characters?
- Would low susceptibility be inherited from parents to offspring making establishment of seed orchards relevant?

Stability of the healthy clones over time

The ability of selected clones to maintain low levels of susceptibility over time was addressed by measuring the clonal seed orchard trials annually from 2007 and forward. Disease symptoms increased in the following years, and most of the clones with a low level of infection in 2007 rapidly developed more symptoms in the following years and the mortality increased. However, at least one (Tree #35; V791) of the 39 clones maintained a low level of symptoms during the following years (Fig. 1B) although surrounded by still more dead or dying trees. Based on the findings we concluded that genetic variation was present and that genotypes with durable resistance therefore are likely to be present in the native ash populations. However, apparently only occurring with low frequency (McKinney et al 2011).

Escape or active defense

Why did some clones not suffer from the disease? The question of potential disease escape versus active resistance became interesting when it was established that the disease was caused by an aggressive wind dispersed pathogen. Especially one observation made in the seed orchard accentuated the question: clones with low levels of susceptibility on average had early leaf yellowing in the autumn suggesting that early senescence was strongly correlated with low level of damage. The correlation was of genetic origin and substantial in magnitude, r_g= -0.79 (McKinney 2011). The correlation was based on observations in 2009, but the same pattern was observed again in 2011 (unpublished results). Other research groups had at that time established that the pathogen infects through the leaves during summer (Gross et al 2012). Thus, it was obvious to speculate that early leaf shed protects the trees by reducing the time available for the pathogen to spread from infected leaves into the twigs and branches of trees.

Potential escape through early leaf shed can have interesting implications, since it indicate that pruning may slow down and limit the development of necroses on infected trees. This of course will need to be studied further. For unmanaged trees it is unlikely that trees would be able to continuously escape from infection, because the peak time of spore dispersal of the pathogen may vary among years according to annual variation in weather conditions. Still, ash trees with early senescence may experience lower levels of infection pressure, and the relationship between phenology and susceptibility is subject for further studies.

Results from two further Danish studies showed that escape from leaf infection cannot be the sole explanation. These studies involved controlled inoculations with the pathogen directly on branches or stems and the results suggested that an active defense must be involved. In 2009, we made controlled infections on braches on a set of eight clones in the TUSE NÆS clonal seed orchard. These eight clones were a subset of the 39 clones, selected to represent varying degrees of susceptibility (McKinney et al 2012). The same year, a second series of controlled infections were made directly on the stem of 123 young trees growing in the HØRSHOLM progeny trial (Lobo et al. 2015). The inoculated trees in HØRSHOLM were single tree offspring of clones from the two old Danish seed orchards (BIRKEMARKEN and GURRE VANG) and the level of susceptibility of their mothers were therefore known from observations in the seed orchards.

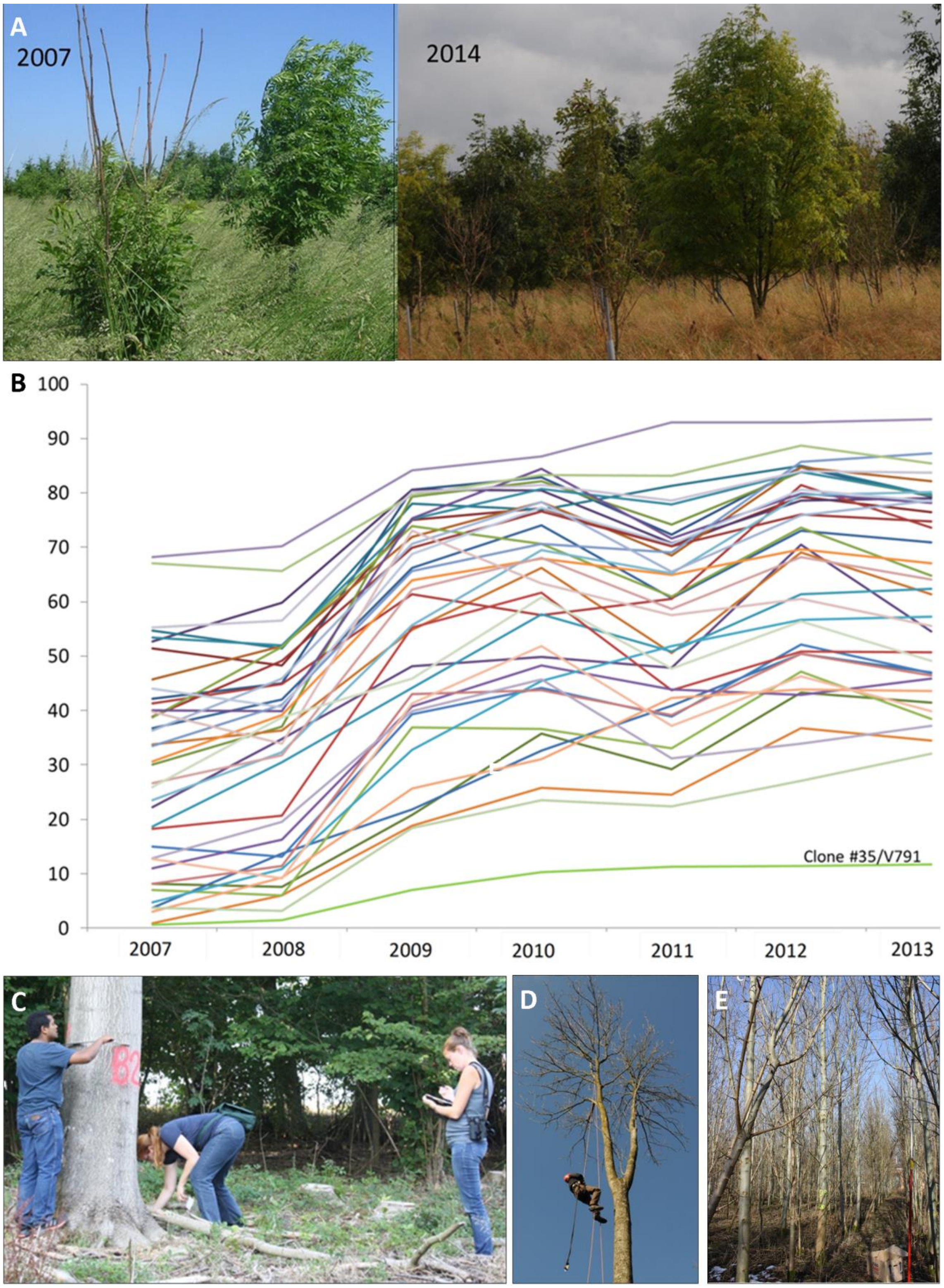


Figure 1 A) Large variation among clones in 2007 (4 years after first report of symptoms) was maintained in 2014. Most trees already exhibited sever symptoms in 2007, but all trees (ramets) of two clones were still completely healthy. TUSE NÆS clonal seed orchard. **B)** Development in average damage level of 39 clones from 2007 to 2013. Slightly modified after McKinney et al. 2014. **C)** Summer assessment and sampling for DNA test of a candidate tree on Bornholm. **D)** Winter collection of scion from a mature candidate tree. **E)** Winter collection of scions from s young candidate tree in HØRSHOLM tree. Photo: A (2007), C, E: Erik Dahl Kjær; A (2014), D: Lars N Hansen.

The development of necrosis around the infection point on branches (TUSE NÆS) or stems (HØRSHOLM) was measured several times in the following year and showed a clear pattern: (1) the lengths of the lesions varied significantly among trees and depended on their genetic background, and (2) variation in necrosis development was closely correlated to susceptibility due to natural infection. The low susceptible clone #35/V791 developed small necroses compared to other infected clones in TUSE NÆS (Fig. 2A; McKinney et al 2012) and the offspring from this clone inoculated in HØRSHOLM also developed small necroses compared to offspring from more susceptible trees (Lobo et al 2015). These results lead us to conclude that the observed differences in susceptibility must be largely due to an active defense in the ash trees. This finding is important, because escape from infection would probably not yield protection in the longer run as discussed above. Further, it opened for the possibility to look for genes involved in the defense mechanism, or genetic markers closely linked to such genes. The findings also showed that necrosis development after controlled inoculation may be developed into a fast and effective quantitative bioassay to predict level of susceptibility of trees grown under field conditions (Fig. 2B).

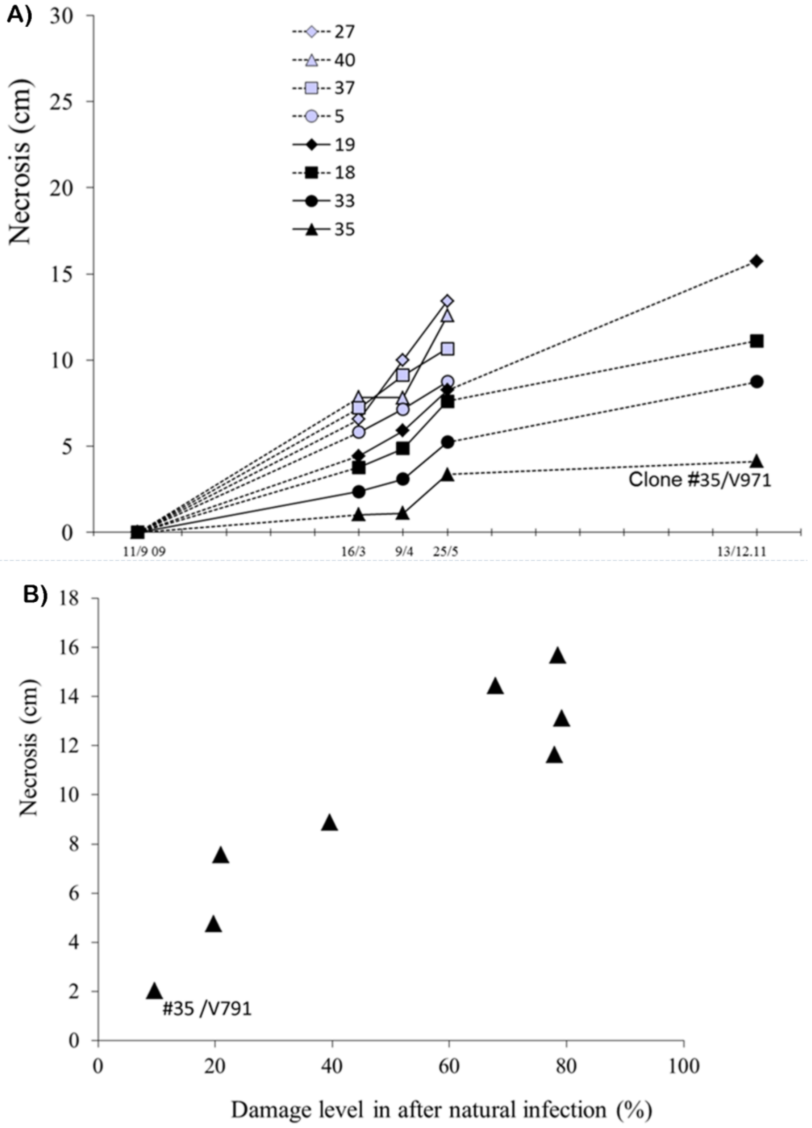


Figure 2 Development of necrosis after controlled infection on branches of eight clones in TUSE NÆS. **A)** Necrosis development of 8 clones during 2 year after artificial infection. **B)** Relationship between necrosis size 6 months after artificial infection and damage level of the 8 clones after natural infection in the TUSE NÆS and TAPSØRE clonal seed orchards. Modified after McKinney et al 2012.

Inheritance from parents to offspring

Inheritance from parents to offspring was estimated from the progenies in RANDERS, SILKEBORG and HØRSHOLM. It was quantified by narrow sense heritability (h_{ns}^2) and additive genetic variation (V_A) estimated from the covariation within and among half sib groups. Moderate to high levels of h_{ns}^2 and V_A were observed and remained as such ($h_{ns}^2 = 0.37$-0.53) during the following years of assessment (Kjær et al 2012; Lobo et al 2014). Simultaneous research in Lithuania provided similar estimates of moderate to high levels of narrow sense heritability (Pliura et al. 2011) supporting that the results reflected a general feature of European ash populations.

The early observation of moderate to high levels of narrow sense heritability was important from a plant procurement point of view, as improved genetic material (with exception of poplars) in all Danish and most other forest tree improvement programs are propagated through seed orchards. The moderate to high levels of h_{ns}^2 also have important implications for management of existing ash forests, because it means that surviving trees will be effective in passing on resistance to their offspring. Surviving, healthy trees left in the forests and landscape may seed abundantly, and thereby procure natural recruitments for next generation with significant improved fitness. It is therefore important to protect such trees. The effect will depend on several factors including level of mortality among susceptible clones, correlation between damage level and fecundity, and the level of gene flow and risk of inbreeding (McKinney *et al* 2014), but the indication led the Nature Agency to rapidly employ a policy of not logging healthy-looking ash trees in State owned forests. Some private forest owners have done the same.

In line with the findings in the clonal trials, the findings from the progeny studies suggested that the frequency of trees with high levels of natural resistance is low, maybe only 1-5%. Most ash trees in existing forests must therefore be expected to be susceptible to the disease. On the other hand most ash stands in the forests are expected to include a few tolerant trees. Presence of genetic variation in susceptibility has also been observed in other cases with high damage from introduced forest tree pathogens, and the frequency of 1-5% is relative high compared to findings from e.g. the Dutch elm disease (Budde *et al* 2016).

It is important to note that the above estimates of narrow sense heritability only refer to young, even-aged stands where the trees have been grown under fairly similar conditions, and where the pathogen poses a high and uniform infection pressure verified as serious and widespread mortality. The heritability may be significantly less in mature stands or scattered groups of mature trees growing in heterogeneous environments. It is the heritability at the mature stage that is relevant for selection of mature candidate trees in the forests for future breeding, but to our knowledge, no estimates for this heritability are present. Although more complicated, it is therefore important to allocate effort into estimation of heritability at this stage.

Selection of candidate trees and development of a breeding program

Based on the accumulated findings from the genetic studies 2007-2009, the Nature Agency in cooperation with private forest owners and the University of Copenhagen initiated a Danish program to identify and propagate healthy trees with the objective to develop seed sources for future restoration of ash forests.

Trees without symptoms were identified with special preference for trees growing in areas where neighboring trees exhibited severe symptoms. The studies from RANDERS and SILKEBORG gave no indication of pronounced provenance variation among the Danish stands in average susceptibility, and by selecting in severely affected stands, we were sure that the candidate trees had been subjected to heavy infection pressure and not simply maintained good health by disease escape.

No sign of symptoms was the major selection criteria, but priority was also given to trees with straight stems and long boles among the healthy trees. Given the limited number of healthy trees, only little attention could however be given to the tree quality. The mobilization phase focused on trees growing in forests, as the most pronounced damage was observed in forests, and the selection pressure therefore assumed to be highest. Also, trees in the forests are at risk of being logged for timber as opposed to landscape trees, which are mainly felled if they show many symptoms.

A special concern was potential relatedness among selected trees. The genetic studies suggested that only a few percent of trees in a stand is expected to possess high level resistance, but that such trees are expected to exhibit their phenotypic superiority with reasonable heritability. Therefore, healthy looking trees growing close together in a single stand could potentially be related. To reduce this risk, we in general only selected one tree per stand and thereby spread the selections across a huge number of Danish stands and forests.

A further concern was that the selected trees could originate from old Danish seed orchards (BIRKEMARKEN, GURRE or TISVILDE), because these orchards have been major seed suppliers to Danish ash plantings since the 1950's. To reduce this risk, preference was made for selection of trees assumed to be older than 55 years. Also, DNA was sampled from all candidate trees to be included in the new seed orchards, and based on their alleles in 4 polymorphic SSR markers we could identify whether any trees had potential origin from the old seed orchards.

Based on the above criteria, forest managers across the country screened forests during the summers of 2012-2014 and identified a large number of mature trees as candidates for selection. The trees were assessed and a subset of 214 trees selected for inclusion in the program (Fig. 3).

The disease causes the most serious symptoms on young trees and, as discussed above, our estimates of heritability strictly speaking only refer to selections among young trees growing under uniform field conditions. Our expectation is that the heritability is lower for selection of mature trees, and that we therefore can obtain maximum response from strong selection in young plantations on uniform site conditions. Thus, the selection of candidate trees in the forests was supplemented with a set of trees selected at a younger age. Especially selection among the thousands of trees in the three progeny trials was an attractive option, as each single tree in these plantings had been assessed for symptoms over several years. Also, they were sampled from a variety of locations and we could control relatedness among selected trees since their exact genetic origin was known. Finally, breeding values for low susceptibility could be estimated based on the performance of their relatives combined with their own phenotype. Based on this approach, 26 outstanding trees were selected out of more than 3500 trees originating from 143 half sib families. The 26 trees selected trees were merge to the pool of trees selected at the mature stage.

Establishment of clonal seed orchards for future seed procurement

Scion material were collected from more than 200 of the selected trees and propagated by grafting. The grafting success varied among the clones, and especially some genotypes from very old trees were lost during the propagation. The health of the grafts was monitored in the nursery, and genotypes that revealed very poor health were also abandoned at this stage.

A total of four clonal seed orchard are to be established 2015/2016. The seed orchards are each based on 35 to 135 clones. The seed orchards with fewest clones include only trees selected on the island of Bornholm, one seed orchard only include trees selected across forests on the island of Sealand, while the two remaining will include more than 100 clones selected across Jutland, Funen and Sealand.

The clonal seed orchards will be established with clones randomized in blocks (random block design) suitable to function as clonal tests until they are ready to carry seed in commercial quantities (expectedly around 2025). The majority of clones will be represented with more than 10 ramets per site.

The clones will be followed with continuous assessments of symptoms during 2015-2025, and clones that do not have sufficiently low levels of infection will be removed prior to the first commercial seed harvest. The seed orchards are designed with a large number of clones planted at a spacing of 12 m^2 per graft. Removal of e.g. 50% of the clones after 10 years will therefore be both technically and genetically feasible. The design allows removal of an even higher fraction of clones in case the efficiency in selection of the mature trees has been lower than anticipated. We expect that additional healthy trees will be identified during the next few years. Thus, some of the seed orchards also include supplementary trees (root stocks) suitable for top-grafting, if additional healthy trees are identified within the next 5 years. These plants (root stocks) are offspring from clone #35/V971 or clone #33/V704.

By 2025, the seed orchards are expected to produce abundant amounts of seed and meet the requirement for restoration and new plantations with ash in Denmark. By that time, we expect that detailed understanding of the genetic background and predicted durability of the resistance has been obtained from the ongoing international research.

Testing and demonstrating superiority of seed from the future seed orchards

The Danish forestry sector has suffered severe losses due to ash dieback, including the substantial costs required for replanting young damaged ash stands with alternative tree species. It is therefore expected that most foresters will be reluctant to plant ash again unless a substantially lower susceptibility of plants from the new seed orchards are demonstrated. Our genetic studies predict that the susceptibility will be markedly reduced due to the strong selection of a few healthy trees in the forests followed by careful testing in the clonal seed orchards that are expected to provide substantial response. However, the gains cannot be verified until the clonal seed orchards are thinned and seeding, and the offspring planted and observed over several years. At the best, such results will be available by 2030.

However, by performing controlled crosses among a limited number of selected and tested trees, we could obtain a plant material that on average should match the future offspring from the selected clones in the seed orchard. Therfore in 2012, a set of clones were crossed. From the clonal trials these clones were known to be among the least susceptible in our test program. Beside healthy x healthy, we also crossed healthy x susceptible to also study segregation in such progenies. Only few clones with documented low level of susceptibility were available at the time, and the crosses were therefore made in cooperation with SKOGSFORSK in Sweden.

Seed were collected from the controlled crosses, pretreated and propagated during 2012-2015. A field experiment will be established in 2016. The trials have been designed to meet the double objective of being a genetic field trial, and simultaneously function as a demonstration site where foresters and restoration managers can observe if offspring from healthy x healthy clones will actually be ‘healthy enough’ to again make the species relevant to plant. The first results from the trial are expected to become available within few years.

New tools to identify resistant trees

The knowledge on genetics of ash dieback is based on observation of variation in phenotypic response of clones or siblings. However, several studies have recently been initiated on the genomic background of the variability of susceptibility and interaction between the host and pathogen. Based on associate transcriptomics, Harper et al. (2016) thus developed and tested a set of SNP (Single Nucleotide Polymorphism) and GEM (Gene Expression Marker) associated with low susceptibility to the Ash dieback pathogen. The markers were identified based on a Danish diversity panel of 200 trees (training set), and subsequently tested on an independent set (test set) of young trees growing in RANDERS but unrelated to any trees in the training set. The results were encouraging. The trees predicted to have a low level of susceptibility based on only four markers were all healthy trees without any symptoms although surrounded by heavily infected trees (Fig. 3). Additional studies in several countries are expected to shed more light on the genetic background of the resistance, which is important in order to predict the likely durability of the observed variation in host susceptibility (Telford et al 2014; Ennos 2014).

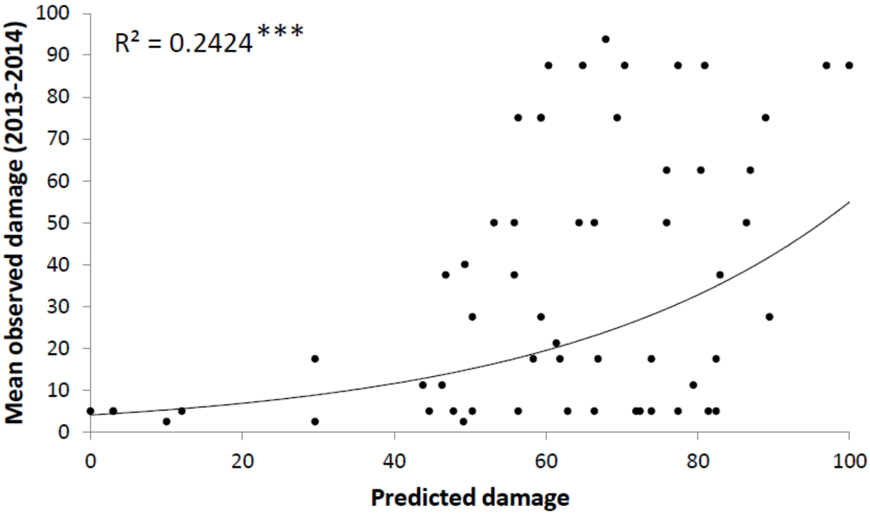


Figure 3 Realized damage level of 66 young tree versus their predicted susceptibility based on 1 SNP and 2 GEM makers. Source: Harper al. 2016.

At present, predictions based on genomic tools have not been implemented in the Danish program, but the rapid progress in development of molecular resources on both the host and pathogen is expected to provide new options to be investigated within the next few years.

The importance of international cooperation

International cooperation has been crucial for the work in Denmark. Cooperation among the Nordic and Baltic countries was facilitated by the SNS supported networks PATHCAR, GENECAR, and ADAPCAR. Ideas, staff, plant material and technical skills were exchanged from 2007 and forward. The cooperation initiated the development of the European FRAXBACK COST network that has facilitated important staff exchange and sharing of information and ideas among European countries. From 2012, cooperation with the partners in the UK based, BBSRC and DEFRA, supported NORNEX consortium has been very important for the development of genomic resources.

The future of ash in Denmark

The genetic findings suggest that the Common ash possesses an evolutionary potential for adaptation to the new emerging disease. Unfortunately, an additional threat is already posed by the Emerald ash borer (Poland & McCullough, 2006) and European ash trees are likely to experience more challenges in the future.

We therefore are dedicated to maintain a high level of genetic diversity in the ash forests, and the Danish program has involved a huge effort to screen many ash populations in order to mobilize a large set of trees to be included in the breeding program. This has only been possible due to the dedicated effort of many public and private forest managers and owners throughout the country. After 10 years of testing and growth we expect that the new clonal seed orchards will be able to procure seed for restoration of ash into the Danish forests.

The requirement for a high level of diversity in the clonal seed orchards (high effective population size in the seed crop) may be in conflict with requirements for development of locally adapted seed sources, because subdividing of the selected trees into several breeding zones will lead to fewer clones per seed orchard. Denmark is a small country where deployment of seed for most woody plant species is based on only one or two zones. Merging the healthy trees into a common breeding population is therefore not expected to cause problems from transfer of germplasm across environmental gradients. However, a special case exists for the island of Bornholm that is geographically isolated from the rest of Denmark and where the ash may have a different genetic origin. Also, the ash is an important species in the native forests. Therefore, a specific seed source for Bornholm has been developed on the initiative of the forest owners from this island, implemented by their forest owners association (Skovdyrkerne) in cooperation with the University of Copenhagen. More studies on the magnitude of local adaptation in ash across Denmark is in progress, where variation in phenology is applied as a proxy for local adaption and genetic markers applied to infer on the levels of gene flow and potential effect of genetic history. Based on the findings, the current strategy can be modified if required.

The presence of additive genetic variation has important implications for management of the native ash forests. The potentially strong selection among a large number of young seedlings in natural regeneration of ash may provide a substantial reduction in susceptibility over a single generation. More research into the response and genetic dynamics of natural populations is required to understand the potential of natural populations to recover. But for the time being it is recommended to maintain healthy ash trees as seed sources for natural regeneration. Also, enrichment planting of offspring from the new seed sources may over time lead to dispersal of higher levels of resistance into exiting old-growth populations through gene flow.

A large number of questions related to wise management and development of the genetic resources still needs to be addressed and the plans for management and restoration of the Danish ash forests will be modified according to new knowledge as such become available. Continued international sharing of results, experience, thoughts and ideas is important. Our expectation is that ash will also be an important species in the European forests in a hundred years’ time.

Acknowledgement

The Danish effort to use natural genetic resistance to restore Danish ash forests is based on a dedicated effort of many people - and we will especially like to recognize the huge effort by many foresters and forest owners who have put knowledge, time and resources into the nationwide screening of ash forests for healthy trees. The research has been supported by grants from GB Hartmanns Fond, The VKR foundation, Nature Agency, and the Danish Research Council. The cooperation within the NORNEX consortium has been supported by BBSRC and DEFRA. We will like to acknowledge the importance of international cooperation that has been supported by EU COST FRAXBACK, and NORDEN SNS GENECAR, ADAPCAR and PATHCAR. A final acknowledgement to Karoline Mythue, Jørgen Andersen, Viggo Jensen and Hans Roulund who established the seed orchards and field trials that have proved so important for the genetic studies.

References

Bakys R, Vasaitis R, Barklund P, Ihrmark K, and Stenlid J, 2009. Investigations concerning the role *of Chalara fraxinea* in declining *Fraxinus excelsior*. *Plant Pathology* 58: 284–92.

Budde, K. B., Nielsen, L. R., Ravn, H. P., and Kjær, E. D. 2016. The Natural Evolutionary Potential of Tree Populations to Cope with Newly Introduced Pests and Pathogens—Lessons Learned From Forest Health Catastrophes in Recent Decades. *Current Forestry Reports* 2: 18-29.

Ennos, R. 2014. Resilience of forests to pathogens: an evolutionary ecology perspective. *Forestry* 88:41–52

Gross A, Zaffarano P, Duo A, Grünig C. 2012 Reproductive mode and life cycle of the ash dieback pathogen *Hymenoscyphus pseudoalbidus*. *Fungal genetics and biology* 49(12):977-86

Harper, AL, McKinney, LV, Nielsen, LR, Havlickova, L, Li, Y, Trick, M, Fraser, F, Wang, L, Fellgett, A, Sollars, ESA, Janacek, SH, Downie, JA, Buggs, RJA, Kjær, ED & Bancroft, I 2016. Molecular markers for tolerance of European ash (*Fraxinus excelsior*) to dieback disease identified using Associative Transcriptomics. *Scientific Reports* 6: 19335.

Kjær, E. D., McKinney, L. V. , Nielsen, L. R. , Hansen, L. N., and Hansen, J. K. 2012. Adaptive potential of ash (*Fraxinus excelsior*) populations against the novel emerging pathogen *Hymenoscyphus pseudoalbidus*. *Evolutionary applications* 5: 219-228.

Kowalski T, Holdenrieder O, 2009. Pathogenicity of *Chalara fraxinea*. *Forest Pathology* 39: 1–7.

Lobo, A., Hansen, J.K., McKinney, L.V., Nielsen, L.R. and Kjær, E.D. 2014. Genetic variation in dieback resistance: growth and survival of *Fraxinus excelsior* under the influence of *Hymenoscyphus pseudoalbidus*. *Scandinavian Journal of Forest Research* 29: 519-526.

Lobo, A., McKinney, L.V., Hansen, J.K., Kjær, E.D. and Nielsen, L. R. 2015. Genetic variation in dieback resistance in *Fraxinus excelsior* confirmed by progeny inoculation assay. Forest Pathology 45: 379–387.

McKinney, L. V., Thomsen, I.M., Kjær, E.D., and Nielsen, L.R. 2012. Genetic resistance to *Hymenoscyphus pseudoalbidus* limits fungal growth and symptom occurrence in *Fraxinus excelsior*. *Forest Pathology* 42: 69-74.

McKinney, L.V., Nielsen, L.R., Hansen, J.K., and Kjær, E.D. 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (Oleraceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. *Heredity* 106: 788-797.

McKinney, L.V., Nielsen, L.R., Collinge, D.B., Thomsen. I.M., Hansen, J.K., and Kjær, E.D, E.D., 2014. The ash dieback crisis: genetic variation in resistance can prove a long term solution. *Plant Pathology* 63: 485–499

Nielsen, L.R., McKinney, L.V., Olrik, D.C., Jensen, V., & Kjær, E.D. 2009, Identity verification of trees in the 61 years old common ash (Fraxinus excelsior) clonal seed orchard FP202 (Birkemarken, Humlebæk) by DNA genotyping with microsatellite markers. *Forest & Landscape Working Papers*, nr. 34/2009. University of Copenhagen.

Olrik, D.C., Kjær, E.D., Ditlevsen, B. 2007. Variation among clones in damage level from Ash Dieback [In Danish]. *SKOVEN* 39 (11): 522-525. Dansk Skovforening, Copenhagen

Pliura, A., Lygis V., Suchockas V., Bartkevicius 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: 17–34.

Poland T.M., McCullough D.G. 2006 Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. *Journal of Forestry* 104:118–24.

Queloz V., Grünig C.R., Berndt R., Kowalski T., Sieber T.N., Holdenrieder O., 2011. Cryptic speciation in *Hymenoscyphus albidus*. *Forest Pathology* 41: 133–42.

Stener, L.-G. 2013. Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* in southern Sweden. *Scandinavian Journal of Forest Research* 28: 205-216.

Telford, A., Cavers, S., Ennos, R.A., Cottrell, J.E., 2014. Can we protect forests by harnessing variation in resistance to pests and pathogens? *Forestry* 88: 3-12.

Overview of ash and ash dieback in Estonia

R. Drenkhan[1]*, A. Agan[2], K. Palm[1], R. Rosenvald[1], L. Jürisoo[1] T. Maaten[1], A. Padari[1], T. Drenkhan[1]

[1] Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Fr.R. Kreutzwaldi, 5; 51 014 Tartu, Estonia, [2] University of Tartu, Institute of Ecology and Earth Sciences, Ravila 14a, 50 411 Tartu, Estonia
** corresponding author: rein.drenkhan@emu.ee*

Abstract

In Estonia, European ash (*Fraxinus excelsior*) grows close to the northern border of its´ natural range in Europe. European ash is a widespread, but not copious deciduous tree species characterized by scattered distribution in mixed forests all over Estonia. In the recent work the health conditions of growing in Estonia 13 different *Fraxinus* species (incl. native European ash) were estimated. In green areas, altogether 416 ash tree individuals were assessed, most numerous species were *Fraxinus mandshurica*, *F. americana*, *F. nigra* and *F. pennsylvanica*. Ash dieback (caused by *Hymenoscyphus fraxineus*) was detected on six different ash species: *F. excelsior, F. nigra, F. pennsylvanica, F. americana, F. mandshurica* and *F. sogdiana.* In forest conditions the worst health of the European ash was estimated in the westernmost monitoring site, where the percentage of healthy trees was only 11% of the total number of estimated trees. At central and eastern monitoring site the percentage of healthy trees in 2013 and 2015 was similarly and constantly 22 %. On the contrary, the higher numbers of healthy naturally regenerated European ash saplings (58%) was registered in the easternmost side of Estonia, compared to the central (40%) and western (44%) monitoring site.

The retention ash trees were analysed after clear-cut. It was established that crowns of retention ash trees in the precut edges (near fields, roads, forest rides) of clear-cuts were less damaged. As trees growing near to the open edges are already acclimated to better light conditions, their vitality and hence resistance to *H. fraxineus* after clear-cut is apparently higher. Also open conditions on clear-cut areas seem to have adverse effect on the ash dieback.

During 6 years (2010-2015) of the research work, the earliest ascocarps of *H. fraxineus* on ash petioles were collected at 5th of June and the latest ones at the 20th of September. In the natural conditions of Estonia the highest number of ascocarps per petiole was countered from the end of July to the end of August. All the fresh and also herbarium specimens (sampled since 1997) were molecularly identified and confirmed to be *H. fraxineus.* In Estonia no evidence, in vivo or in herbaria could be stated for *H. albidus.*

Key words: *Hymenoscyphus fraxineus*; health conditions of *Fraxinus* spp.; exotic ash species; natural regeneration of European ash; retention trees

Introduction

Importance of ash trees in Estonia

European ash (*Fraxinus excelsior* L.) is a widespread deciduous tree species characterized by scattered distribution in mixed forests all over the total forested area of Estonia (Valk and Eilart 1974, Laas 1987, Kukk and Kull 2005). In Estonia, European ash grows close to the northern border of its´ natural range in Europe. As a main forest tree species, *F. excelsior* covers only 0.33% of the total forested area, but ash volume in the total volume of tree species is 1% (Raudsaar et al. 2014). The abundance of European ash in mixed stands decreases in the direction from the northwest to southeast. Pure European ash stands are quite rare. Natural regeneration is the predominant way of European ash regeneration (Valk and Eilart 1974).

In forest management practice the healthy ash trees are harvested only in stands where ash is the dominant species. Following the forest management rules (see the Forest Act) the rotation age of ash stands is dependent on the forest site type quality classes and varies between 90 – 130 years (Forest management rules 2006). In other cases ash trees are left as seed or/and retention trees during the clear-cut. More precisely it is stated in the Forest Act as follows: In the event of clear cutting, all trees are cut from the stand within one year after the beginning of the cutting, with the exception of: 1) 20–70 *Pinus, Betula, Fraxinus, Quercus, Alnus glutinosa, Ulmus* spp. per hectare, dispersed or situated in small groups, which are left as seed trees, and viable advanced regeneration (The Forest Act). Such legal status is important bases for conserving the European ash as a sustainable species in Estonian forests. The majority of ash dominated stands are naturally occurring whereas artificial regeneration by the use of ash plants or seedlings has been marginal. The last year when ash plants were produced in Estonian nurseries was 2004, and even then by the official statistics only with the marginal production – 3000 plants. During the last 20 years, the highest number of ash plants (71 000 plants) was produced in 1998. For example, total production of seedlings and plants of forest trees in Estonian nurseries was 51.27 million (in 2011, including plants for export). During 1979 – 2004, seeds of European ash used in nurseries were collected from stands of the category 'source identified' of the EC Directive 1999/105/EC on the marketing of forest reproductive material. There are no statistics about the usage of a crop from the 0.1 hectare ash seed orchard, established in 1963 (Kurm and Tamm 2000, Tetlov et al. 2014, Valk and Eilart 1974). There have never been any breeding programs, provenance trials, or genetic studies for European ash in Estonia. In Estonia on the basis of site conditions have been distinguished two different ecotypes of European ash – the floodplain and the limestone ash (Laas 1987, Valk and Eilart 1974). In Europe the real existence of such ecotypes has never been proven by progeny studies (Pliura and Heuertz 2003).

Importance of ash trees in urban areas in Estonia

The European ash had been popular as an ornamental tree, planted as single trees or small groups in green areas all around Estonia (Kuusk et al. 1996). However, different *Fraxinus* species are less used in urban areas where air pollution is higher; they also need good site conditions. On the other hand, their leaves come out late, fall early, and their root systems can be aggressive (Laas 1987, Sibul 2007), and their attractiveness is decreasing. European ash is found in most historical parks in Estonia, very often representing the thickest and tallest single trees there or forming small groups in parks or green areas (Abner et al. 2007). Ash trees in rural areas are often been favoured as neighbours, growing close to the farmhouses. During the recent research there are identified 13 ash species (incl. the native *Fraxinus excelsior*) in open landscapes, parks and other types of green areas in Estonia.

Importance of ash trees for biodiversity

The high biodiversity value of ash trees is well known. A large number of organisms are related to ash trees, e.g. the epiphytic lichens and bryophytes, wood-decaying fungi and saproxylic insects. Many species are specifically dependent on ash trees (review listed e.g. in Pautasso et al. 2013). In Estonia, mainly lichens growing on ash trees have been studied, and the results confirm that ash trees, especially large ash trees, provide a habitat for many epiphytic lichen species (e.g. Jüriado et al. 2009, Thor et al. 2010, Lõhmus and Runnel 2014). The species richness of the genus *Fraxinus* is one of the highest among the tree species in Estonia (Jüriado et al. 2009, Lõhmus et al. 2012). The highest number of red-listed species in wooded meadows is recorded on ash trees (Thor et al. 2010). The loss of decrease of ash trees in Estonia from ash dieback disease would result in a great loss of biodiversity. Of the many causes of biodiversity loss already taking place in Europe, ash dieback would be among the worst (Pautasso et al. 2013).

Health conditions of ash trees and the disease history

Ash dieback (caused by *Hymenoscyphus fraxineus*) is a fast grow serious forest pathological problem in Europe. The health conditions of European ash decreased evidently since the early 1990s starting in north-eastern Poland (Kowalski 2001). Since then the fungus was distributed to different European countries (Gross et al. 2014). *H. fraxineus* may originate from Far-East Asia (Zhao et al. 2013).

There is firm evidence in Estonia that *Fraxinus mandshurica*, originating from Far-East Russia, was planted in some manor parks in the beginning of 20th century or earlier, as manor owners had botanical interest (Berg 1924, Sander 2000). Many of those trees did not survive (Drenkhan et al. 2014), apparently because young *F. mandshurica* trees suffer from the late frosts (Laas 1987). From the 1960s to the 1980s, seeds and plants of *F. mandshurica* were introduced to Estonia and a few trees are still alive (Drenkhan et al. 2014).

The aim of this paper is to compose an overview of the recent health situation research of mature ash in Estonia, native and exotic, the natural regeneration of European ash and recent and ongoing research concerning ash dieback disease.

Health conditions of natural regeneration of European ash

The estimation of the health of natural regeneration of European ash was carried out on a West – East transect through Estonia: in Lääne, Viljandi, Jõgeva counties (Fig. 1). Data for the current study were collected on the same monitoring sites at 2013 and 2015, except of some differences in the Läänemaa site. Six monitoring plots (stands or clear-cuts) were chosen randomly in each monitoring area, where European ash was found forming 50% or more in the composition of tree species on the stands and clear-cuts (before cut). Generally, three 100 m^2 monitoring plots were randomly chosen and located per sample site per hectare. In total 51 (in 2013) and 46 (in 2015) monitoring plots were measured on these 3 monitoring sites (Fig. 1). Within each monitoring site all ash trees were found naturally regenerated. The majority of monitoring plots were located in eutrophic deciduous forests (*Aegopodium* site type according to Lõhmus, 1984). Ash saplings were counted and estimated into different health classes on every monitoring plot. Health classes were divided into four categories: healthy (saplings with no visual symptoms of ash dieback), diseased (symptomatic saplings of ash dieback), injured (saplings damaged by other factors, like herbivores) and dead. Also fruiting bodies or rhizomorphs of *Armillaria* species were sampled. Statistical calculations were carried out using program R: T-test and Principal Component Analyses.

Figure 1 Monitoring sites on West–East transect through Estonia: in Läänemaa, Viljandi and Jõgeva counties. Systematically collected ascocarps of *H. fraxineus* from Nõgiaru permanent sampling site.

In 2013 and 2015 total of 2367 and 1591 naturally regenerated saplings, respectively, were counted and estimated on monitoring sites. The mean number of saplings per monitoring plot was 46 and 35 in 2013 and 2015, respectively. Number of healthy seedlings was higher in 2013 compared to 2015, 51% and 41%, respectively. The amount of diseased saplings in different years was 41% and 53%, respectively (see Fig. 2). Statistical analyses showed that no statistically significant differences existed between number of healthy and diseased saplings in these two years, although the number of healthy seedlings decreased in 2015 (Fig. 2).

The health status of ash saplings was also compared between the seedlings growing on clear-cut areas (N=52 monitoring plots) and understory seedlings (N=40), calculation was done for the total dataset, collected during 2013 - 2015. From total number of estimated saplings was 41% on clear-cut and 53% on understory monitoring sites. The numbers of healthy saplings were similar on clear-cut and understory areas. The number of diseased saplings was 19% higher in understory sites compared to clear-cut areas, $P<0.05$. It may indicate that under the mature ash trees there are better conditions for the infection of saplings or for the pathogen development than in open areas. On the other hand, saplings on understory sites are located in a higher stress conditions, e.g. suffering for limited nutrients, light, etc.

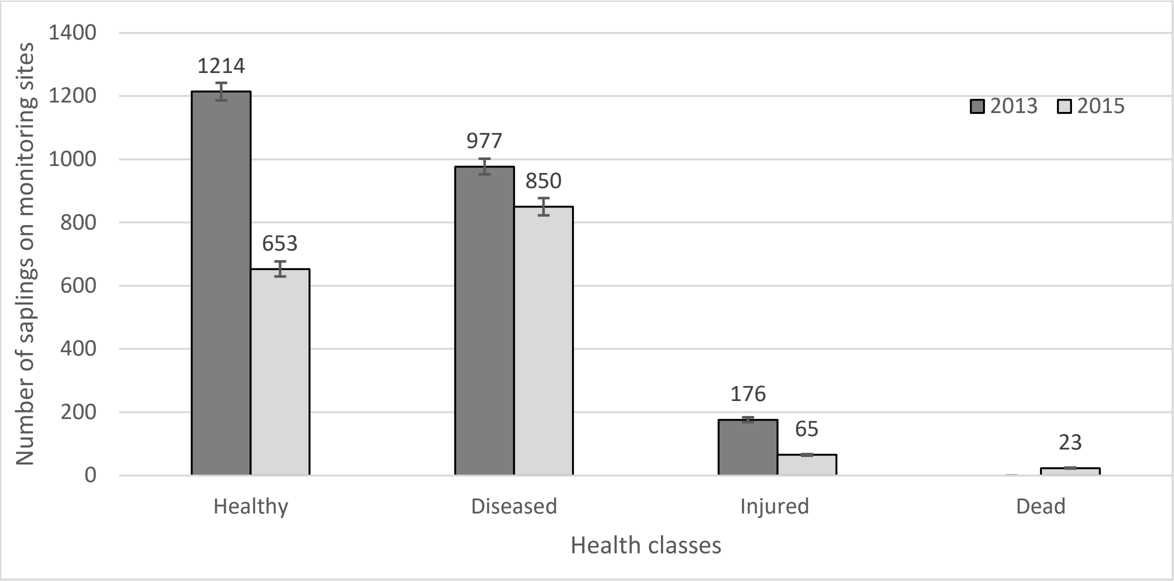


Figure 2 Health condition of naturally regenerated ash saplings at 2013 and 2015. Number of samplings on monitoring plots is presented in top of the columns per health classes and years. Error bars indicate the standard deviation values.

In all monitoring sites, there were similar numbers of saplings registered during 2013 - 2015. Surprisingly, in the most eastern monitoring site (in Jõgeva county) there was statistically significantly higher number of healthy saplings (58% of saplings were healthy) than in Viljandi (40%) and Lääne (44%) counties. The highest number of saplings was registered in the westernmost side of Estonia, in Läänemaa county (54%) than in Viljandi (52%) and Jõgeva (31%) counties. This is known that the ash dieback symptoms were first time noted in north-western Estonia in beginning of 2000s and the disease has moved east (Drenkhan et al. 2014). According to these data we cannot confirm any decreasing trend in development of the epidemic. In all monitoring sites the number of injured (damaged by herbivores) samplings was higher in 2013 (7.4% of seedlings) than in 2015 (4.1% of samplings), but no statistically significant differences were found.

Health conditions of mature ash trees in forests

The health conditions of mature trees was visually estimated on three monitoring sites on a West–East transect through Estonia during 2013 – 2015 (see Fig. 1). The worst condition was estimated in Lääne monitoring site, where the percentage of healthy trees in 2013 was 11% of total number of trees (N=118). At Viljandi and Luua the percentage of healthy trees (22 %) was comparable in 2013 (N=97) and 2015 (N=72).

In another research, the health status and mortality of altogether 99 ash trees was measured in (over)mature forests of south-eastern Estonia in 2009-2013 (Lõhmus and Runnel 2014). The ash trees populations of studied forests declined dramatically from 2009 to 2012, but only minor changes occurred in year 2013. By 2012, almost half of the overstory trees were dead, and the proportion of trees with <50% crown lost was only 21%. The subcanopy ash trees showed slightly better survival and vitality (Lõhmus and Runnel 2014).

Health conditions of retention ash trees on clear-cut areas

The survival and vitality of European ash retention trees (altogether 577 trees) was monitored on 41 clear-cut (retention cut) areas located in central and eastern Estonia and cut in 2001-2002, i.e. before the ash dieback epidemic started (Rosenvald et al. 2015). By the year 2014, 65 percent of ash retention trees were alive; while 15% of assessed trees were externally healthy. The vitality of ash trees was evaluated since 2009, in different categories based on position of retention trees on the area. The trees retained in the precut edges (near fields, roads, forest rides) had the highest vitality, and among them were recorded 32% of healthy trees in 2014. The vitality of ashes in postcut edges (near forest) was high at the beginning of study (2009), but decreased in time, and by the 2014, the percentage of healthy trees fell to 13%. The lowest vitality was recorded in ash trees left in central parts of cuts; only 10.5% healthy by 2014. Also the analysis of the crown decline between 2009 and 2014 confirmed that trees suffer most near postcut edges and least near precut edges, and the decline was moderate on trees in centre of clear-cut area. The result indicates that ash dieback intensity is related to physiological stress of trees, and the acclimation process plays an important role in the health of ash trees in postharvest stands. Also the open conditions on clear-cuts seem to have an adverse effect on ash dieback (Rosenvald et al. 2015).

The presence (or absence) of *H. fraxineus* was detected from the petioles, originated from 33 visually healthy ash trees, in autumn 2013. The results demonstrated that all of the petioles, and hence all of the trees, were infected by *H. fraxineus*. Hence, the infection of *H. fraxineus* may not always influence the vitality of the host tree (Rosenvald et al. 2015). In the same study, *Armillaria* spp. was examined from wood samples of 102 trees; 28 of them were infected. Although, it could be assumed that trees with more damaged crowns are more likely sensitive to *Armillaria* infection (*Armillaria*, as generally known, strikes weakened trees), in this study, the damage of crowns seemed not to be related to damage of roots (Rosenvald et al. 2015).

Health conditions of ash trees in green areas in Estonia

Specification and finding of study areas was carried out using data from many different publications (Laas 1987, Elliku et al. 1997, Sander 1999, 2001), and during the dendrological inventory of parks – either manor parks or urban ones. Sixteen of 23 *F. mandshurica* habitats were examined, noted in a previous research (Drenkhan *et al.* 2014).

An important purpose of the field work was to identify the locations of the trees and habitats mentioned in our sources. This task was a challenge, given the vague and sometimes inaccurate information about many of the actual growth locations. Varieties and cultivars were not separately identified. After a tree was located and identified, its health condition was visually assessed. The main focus was any symptoms of ash dieback. Other factors that could affect tree health, such as frost cracks and other fungal infections, were also marked on the worksheet. Tree height, diameter of the root collar and diameter of the trunk at 1.3 meters above the ground were measured. The average diameter of the root collar was calculated. Crown diameters were measured, but this data was not analysed in this work. Four vitality classes, determined by visual assessment for native and exotic ash species, were identified (Rosenvald *et al.* 2015): 1, healthy (sound-looking, no dried branches in the crown); 2, branch loss (SDB, some branches dead and/or up to one quarter of the crown consists of dried branches); 3, damaged (HCD, many branches dead and/or about half of the crown consists of dried branches); 4, dead or dying (none/one or two live branches remained).

In total, 415 ash trees in Estonia were assessed, incl. twelve different exotic ash species (together with European ash, see Fig. 3 and Table 1), the most numerous of which were *Fraxinus mandshurica* (representing the East Asian ash species) and *F. americana, F. nigra, F. pennsylvanica* (the North American ones, respectively). The rest of species (of different origins) was represented by a single or by two individual trees. As a result, *H. fraxineus* was detected in Estonia on six different ash species: *F. excelsior*, *F. nigra*, *F. pennsylvanica*, *F. americana*, *F. mandshurica* and *F. sogdiana* (see Drenkhan and Hanso 2009, 2010, Rytkönen et al. 2011, Drenkhan et al. 2015).

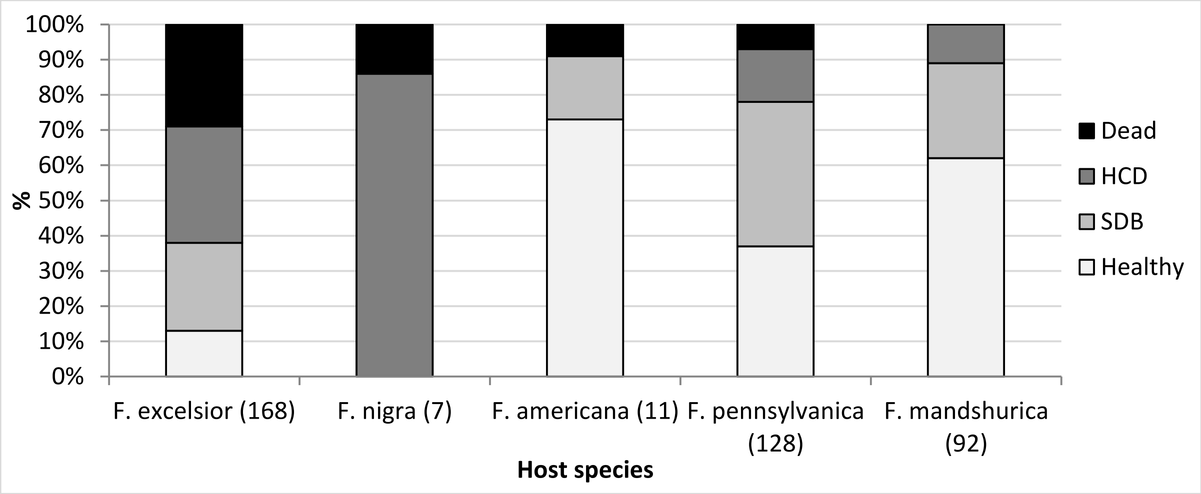


Figure 3 The percentage of ash species of different health conditions (SDB - single dead branches in crown, HCD-half of the crown dead). Number of estimated trees is presented after host name in parenthesis.

Table 1 List of estimated Fraxinus species health condition in Estonia during 2013-2014 (SDB - single dead branches in crown, HCD - half of the crown dead). Less than five individuals observations are given in parentheses.

Host species	Number of trees	Healthy, %	SDB, %	HCD, %	Dead or dying, %
Fraxinus americana	11	73	18	0	9
F. chinensis	2	(100)	(0)	(0)	(0)
F. excelsior	168	13	25	33	29
F holotricha	1	(0)	(100)	(0)	(0)
F. latifolia	1	(0)	(0)	(100)	(0)
F. longicuspis	1	(100)	(0)	(0)	(0)
F. mandshurica	92	62	27	11	0
F. nigra	7	0	0	86	14
F. ornus	1	(100)	(0)	(0)	(0)
F. paxiana	1	(0)	(100)	(0)	(0)
F. pennsylvanica	128	37	41	15	7
F. potamophilia	1	(0)	(0)	(0)	(100)
F. sogdiana	1	(0)	(100)	(0)	(0)
Total number of trees	415				

In several cases it was difficult to find a certain connection between the ash dieback and declining of trees. It was also problematic in this study as most of the found and investigated introduced ash species were represented only by one or two individual trees (see Table 1). It also makes the objective evaluation and comparison of different species difficult as there is no way to compare the same ash species growing in different habitats. There may be a lot of other reasons why one individual tree is under stress, for example genetic variability, unsuitable environmental condition, lack of light, less nutrients in the soil, etc.

The results of this study show that the most healthy ash species in Estonia were *F. mandshurica, F. chinensis, F. longicuspis*, and *F. ornus*. It is known from earlier studies that *F. ornus* is quite resistant to *H. fraxineus*, although some infection is possible (Kräutler and Kirisits 2012). There is also a lot of information about *F. chinensis* and it was estimated resistance towards ash dieback in its natural habitat in China and in countries where it has been introduced (Gross et al. 2014, Zheng and Zuang 2014). Less was known about the ash dieback on *F. longicuspis* and the resistance of that host towards the disease. Ash species that had only some few dead branches were *F. sogdiana, F. paxiana* and *F. holotricha*. Despite having only a few dead branches, the further analyses showed that *F. sogdiana* was infected by *H. fraxineus* (Drenkhan et al. 2015). There was no previous information about *H. fraxineus* infection on the named above ash species. Only one specimen identified as *F. latifolia* was found growing in Estonia (in western Estonia, Saaremaa). Its crown was already half dead but on this host species *H. fraxineus* was not found. The artificial inoculation of this particular species with *H. fraxineus* did not result in any necrotic lesions (Gross et al. 2014). In our study we also did not succeed to isolate *H. fraxineus* from *F. ornus* and we cannot conclude that that the poor condition of this tree species was caused namely by *H. fraxineus*.

A dead *F. potamophila*, which was found in Saaremaa, had distinct symptoms of ash dieback. Abundant fruiting bodies of *H. fraxineus* on the last year's petioles were present under the tree, a clear indication of ash dieback. Unfortunately, *H. fraxineus* from a shoot was not isolated. No previous evidence that *H. fraxineus* has been documented on *F. potamophila.*

In Estonia, *H. fraxineus* had earlier been isolated from *F. americana, F. mandshurica, F. nigra* and *F. pennsylvanica*, among which *F. nigra* seemed to be most harmed (Drenkhan and Hanso 2010). As in our studies the crowns of six trees of *F. nigra* out of the examined seven were half-dead and one was fully dead, showing the same tendency of low resistance to the disease. On the other hand, all other ash species which originated from North America had relatively little damaged.

A large majority of *F. mandshurica* trees growing in Estonia were in relatively good health conditions. This means that the health status of *F. mandshurica* specimens was seemed unchanged since they were assessed in good shape in 2009 (cf. Drenkhan and Hanso 2010), most of them were in good shape.

In conclusion, some species, which had the same high damage rate as *F. excelsior*, were *F. nigra* and *F. potamophila*. *F. americana*, *F. pensylvanica* and especially *F. mandshurica* were quite healthy: from them 73%, 38% and 62%, respectively, evaluated as healthy and 18%, 40% and 32%, respectively, having only single dead branches (Fig. 3). For the few number (1-2 specimens) of some other ash species, we could not make any trustworthy conclusions for comparison of those species with the European ash. It is necessary to continue the monitoring and analyses of the health of all exotic ash species, especially *F. potamophila* and *F. longicuspis* in order to precisely identify the causal agent of damage.

Phenology of *H. fraxineus* reproduction in Estonia

From 2010 to 2015, the ascocarps of *H. fraxineus* (Fig. 4) were systematically collected from ash stand on Nõgiaru sample plot (see Fig. 1), Tartu county, southern Estonia. Petioles with ascocarps that fell the previous year were randomly collected during the vegetation period. The collection of samples was done during the vegetation period once per a week. In the lab, the mean numbers of ascocarps per petiole was counted, and the mean number of ascospores per fruiting body for the different sampling dates was calculated. The purpose was to estimate the number of emerging ascocarps and to identify the sporulation time of ascospores during the vegetation period.

Figure 4 Ascocarps of *Hymenoscyphus fraxineus* on a previous year petiole of European ash on the ground (photo: R. Drenkhan).

The earliest ascocarps during 6 years of research work were collected at 5th of June, 2013 and latest ones at the 20th of September, 2011. The highest number of ascocarps was counted from the end of July to the end of August. At the same time, the highest number of ascospores was measured morphologically under microscope from the end of July to mid of August, but it depended strongly on weather conditions at the sampling year. For example, in Norway active release of pathogen ascospores was measured in the morning and it peaked from mid-July to mid-August (Hietala et al. 2013). In the conditions of southern Estonia, the ascocarps of *H. fraxineus* could be found on petioles of European ash during 81 – 85 days.

Search of *Hymenoscyphus albidus* in Estonia

In 2010, fallen ash petioles carrying apothecia of *Hymenoscyphus* sp. were sampled from five different areas on a northwest-southeast transect through Estonia: Rapla → Sürgavere → Ülenurme, Nõgiaru and Tähtvere and identified by molecular methods (Drenkhan et al. 2012). *H. albidus* was not found, only *H. fraxineus*, without exception, was registered (Drenkhan et al. 2012, Drenkhan et al. 2014). Similarly, in Polish ash stands the saprotrophic *H. albidus* was recently completely absent (Kraj and Kowalski 2014) and it was the same case in some other eastern European countries (McKinney et al. 2012; Baral and Bemmann 2014). Has *H. albidus* ever occurred in Estonia, remains unknown also after the investigation of herbarium specimens.

There is some ambiguity between the herbarium evidence (i.e. of dating of the first samples of *H. albidus* vs. *H. fraxineus* in herbaria) and later comments concerning the basic question: the earliest occurrence of the pathogen in Estonia (Drenkhan et al. 2016). The oldest deposited specimen of the Baltic area identified as *Hymenoscyphus albidus* (Roberge ex Desm.) W. Phillips (TAAM 44226) was sampled at 16.07.1966 in Marijampol, Lithuania. All other *Hymenoscyphus* specimens on ash petioles sampled since 1997 and deposited in fungal collection in Estonia (TAAM in Tartu) were originally identified as *H. albidus*. These herbarium and all other fresh specimens were molecularly confirmed and indicated to be *H. fraxineus* (Drenkhan et al. 2016).

Management options to mitigate the impact of ash dieback

Silvicultural management options

At the moment, there is no single solution to ash dieback disease. However, some tools can help to mitigate the development of the disease, and improve conditions for ash trees to exist in the future. We point out four of them:

1) *Genetic resistance.* The apparently most perspective solution against ash dieback seems to be introducing genetically resistant trees (Gross et al. 2014). Although in the nature the genetically resistant ash trees are quite rare (ca 5% from all ashes; reviewed in Rosenvald et al. 2015); those trees can be a source for the creation of new resistant generations for the future. Hence, in case of clear-cutting, visually healthy ash trees must be retained as retention trees (Rosenvald et al. 2015).

2) *Enhancing resistance of ash trees through the harvest.* Less competition and favourable conditions (enough light, nutrients and water) enhances the vitality of ash trees (Rosenvald et al. 2015). Favourable growth conditions enhance the survival potential of genetically resistant ash trees but also trees with medium resistance to ash dieback (the resistance to *H. fraxineus* is not absolute). Ash retention trees near open areas (including roads, forest rides and ditches), but also the trees having long crowns (which indicates the light acclimation) are potentially more resistant (Rosenvald et al. 2015).Visually healthy trees should be retained by selection cutting and thinning practice, but the effectiveness of this action for promoting the long-term vitality of trees needs more studies (Bakys et al. 2013, Rosenvald et al. 2015).

3) *Specific plant protection methods.* One, although still theoretical perspective is the biological control of *H. fraxineus* by the use of mycoviruses (Schoebel et al. 2014) or other microorganisms. From chemical treatments, the use of urea prevents apothecium formation on collapsed ash leaf petioles, and thus prevents the spread of the pathogen (Hauptmann 2015). The use of fungicides (Dal Maso et al. 2014) and heat treating of ash seedlings (Hauptman et al. 2013) slows down the development of *H. fraxineus*, although it is not applicable in practical large-scale forest management in Estonia.

4) *Import Fraxinus spp. seeds and seedlings*. Inspection of imported ash seeds and seedlings to avoid the introduction of new pathogen lineages and subsequent formation of genetically new virulent strains of *H. fraxineus*.

Should we use some alternative, less susceptible to dieback Fraxinus species?

We do not recommend a large-scale replacement of European ash in Estonia with more resistant ash species. The addition of resistant and decorative ash species may be considered for parks and avenues, but should not be extrapolated to the forests. Ash easily hybridizes, which would pose a danger for the future of the European ash. When the current phase of climate change has passed, it is possible that the health condition of European ash will recover again.

Acknowledgements

The study was supported by the Estonian Environmental Investments Centre, the Norwegian Financial Mechanism 2009-2014 under the project EMP162, by the Institutional Research Funding IUT21-04 and by COST action FP1103 (FRAXBACK). Guy Meilleur is appreciated for the language revision.

References

Abner, O., Konsa, S., Lootus, K. and Sinijärv, U. 2007. Eesti pargid 1 [Estonian Parks 1]. Tallinn: Keskkonnaministeerium, Muinsuskaitseamet, Varrak. Edited by Konsa, S., Sinijärv U., 400 p. (In Estonian)

Abner, O., Konsa, S., Lootus, K. and Sinijärv, U. 2012. Eesti pargid 2 [Estonian Parks 2]. Tallinn: Keskkonnaministeerium, Muinsuskaitseamet, Varrak. Edited by Konsa, S., Sinijärv U., 640 p. (In Estonian)

Bakys, R., Vasaitis, R., Skovsgaard, J.P. 2013. Patterns and severity of crown dieback in young even-aged stands of European ash (*Fraxinus excelsior* L.) in relation to stand density, bud flushing phenotype and season. Plant Prot. Sci. 3: 120-126.

Baral, H.-O., Bemmann, M. 2014. *Hymenoscyphus fraxineus* vs. *Hymenoscyphus albidus* – a comparative light microscopic study on the causal agent of European ash dieback and related foliicolous, stroma-forming species. Mycology 5(4): 228-290

Berg, F. 1924. Tree orders in the forest and park of Sangaste. *Eesti Mets* 15/16: 157-161; 17/18: 177-180. (In Estonian)

Dal Maso, E., Cocking, J., and Montecchio, L. 2014. Efficacy tests on commercial fungicides against ash dieback *in vitro* and by trunk injection. *Urban Forestry & Urban Greening* 13: 697-703. doi:10.1016/j.ufug.2014.07.005.

Drenkhan, R. and Hanso, M. 2009. Saaresurm Eestis ja mujal Euroopas [Decline of European ash in Estonia and elsewhere in Europe]. *Eesti Loodus (Estonian Nature)* 60: 14-19. (In Estonian)

Drenkhan, R. and Hanso, M. 2010. New host species for *Chalara fraxinea*. *New disease reports* 22: 16.

Drenkhan, T., Drenkhan, R. and Hanso, M. 2012. Saaresurma tekitaja on hoopis teine, invasiivne kottseen [The causal agent of ash dieback is actually another, invasive *ascomycete*]. *Eesti Loodus (Estonian Nature)* 63: 46-48. (In Estonian)

Drenkhan, R., Sander, H. and Hanso, M. 2014. Introduction of Mandshurian ash (*Fraxinus mandshurica* Rupr.) to Estonia: Is it related to the current epidemic on European ash (*F. excelsior* L.)? *European Journal of Forest Research* 133: 769–781. doi: 10.1111/ efp.12110.

Drenkhan, R., Adamson, K., and Hanso, M. 2015. *Fraxinus sogdiana*, a Central Asian ash species, is susceptible to *Hymenoscyphus fraxineus*. *Plant Protection Science* 51: 150-152.

Drenkhan, R., Riit, T., Adamson, K. and Hanso, M. 2016. The earliest samples of *Hymenoscyphus albidus* vs. *H. fraxineus* in Estonian herbaria and the development of new species-specific PCR primers. *Mycological Progress* (Submitted)

Elliku, J., Paivel, A. and Sander, H. 1997. Saaremaa looduskaitsealuste arboreetumiter ja parkide dendrofloora (1952-1996). [Woody Plants of Arboretums and Parks Under Nature Conservation of Saaremaa Island (1952-1996)]. Tallinn. 65 p.

Forest Management Rules 2006. Estonian Minister of Environment [https://www.riigiteataja.ee/akt/126022014016?leiaKehtiv) (In Estonian)

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V. and Sieber, T.N. 2014. *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. Pathogen profile. *Molecular Plant Pathology* 15: 5-21. doi:10.1111/mpp.12073.

Jüriado, I., Liira, J., Paal, J. and Suija, A. 2009. Tree and stand level variables influencing diversity of lichens on temperate broad-leaved trees in boreo-nemoral floodplain forests. *Biodiversity and Conservation* 18: 105-125. doi: 10.1007/s10531-008-9460-y.

Hauptman, T., Piškur, B., de Groot, M., Ogris, N., Ferlan, M. and Jurc, D. 2013. Temperature effect on *Chalara fraxinea*: heat treatment of saplings as a possible disease control method. *Forest Pathology* 43: 360-370. doi:10.1111/efp.12038.

Hauptman, T., Celar, F.A., de Groot, M. and Jurc, D. 2015. Application of fungicides and urea for control of ash dieback. *iForest* 8: 165-171. doi:10.3832/ifor1272-008.

Hietala, A.M., Timmermann, V., Borja, I. and Solheim, H. 2013. The invasive ash dieback pathogen *Hymenoscyphus pseudoalbidus* exerts maximal infection pressure prior to the onset of host leaf senescence. Fungal Ecology 6: 302-308.

Kowalski, T. 2001. About the dying of ash trees. *Trybuna Leśnika* 4: 6–7. (In Polish.)

Kraj, W., Kowalski, T. 2014. Genetic variability of *Hymenoscyphus pseudoalbidus* on ash leaf rachises in leaf litter of forest stands in Poland. Journ Phytopathol 162: 218-227.

Kräutler, K; Kirisits, T. 2012. The ash dieback pathogen *Hymenoscyphus pseudoalbidus* is associated with leaf symptoms on ash species (*Fraxinus* spp.). *Journal of Agricultural Extension and Rural Development*, 4, 14 May 2012, 261-265.

Kukk, T. and Kull, T. 2005. Eesti taimede levikuatlas [Atlas of the Estonian Flora]. Tartu, 528 p.

Kurm, M. and Tamm, Ü. 2001. Deciduous trees in Estonia. In H. Tullus and A. Vares (Editors), Proceedings of the Estonian Academical Forestry Society XIV. Silviculture of deciduous in Estonia. 24-67.

Kuusk, V., Tabaka, L. and Jankevičiene R. (eds) 1996. Flora of the Baltic countries. II. Estonian Acad Sciences. Instit Zool Bot, Latvian Acad Sciences, Instit Biol, Lithuanian Inst Bot. Eesti Loodusfoto AS, Tartu

Laas, E. 1987. Dendroloogia [Dendrology]. Tallinn, Valgus, 824 p.

Lohmus, E. 1984. Eesti metsakasvukohatuubid. [Estonian forest site types.] Eesti NSV Agrotoostuskoondise Info- ja juurutusvalitsus, Tallinn, Estonia.

Lõhmus, A. and Runnel, K. 2014. Ash dieback can rapidly eradicate isolated epiphyte populations in production forests: A case study. *Biological Conservation* 169: 185-188. doi:10.1016/j.biocon.2013.11.031.

Lõhmus, P., Leppik, E., Motiejunaite, J., Suija, A. and Lõhmus, A. 2012. Old selectively cut forests can host rich lichen communities - lessons from an exhaustive field survey. *Nova Hedwigia* 95(3-4): 493-515. doi: 10.1127/0029-5035/2012/0064.

McKinney, L. V., Thomsen, I. M., Kjær, E. D., Nielsen, L. R. 2012. Genetic resistance to *Hymenoscyphus pseudoalbidus* limits fungal growth and symptom occurrence in *Fraxinus excelsior*. Forest Pathology, 42(1), 69-74.

Pautasso, M., Aas, G., Queloz, V. and Holdenrieder, O. 2013.European ash (Fraxinus excelsior) dieback - A conservation biology challenge. *Biological Conservation* 158: 37-49. doi:10.1016/j.biocon.2012.08.026.

Pliûra A., M. and Heuertz, M. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for common ash (Fraxinus excelsior). International Plant Genetic Resources Institute, Rome, Italy, 6 p.

Raudsaar, M., Pärt, E. and Adermann, V. 2014. Forest Resources. In Yearbook Forest 2013. Estonian Environment Agency, 1-42.

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(9): 1206-1214. doi: 10.1139/cjfr-2014-0512.

Rytkönen, A., Lilja, A., Drenkhan, R., Gaitnieks, T. and Hantula. J. 2011. First record of *Chalara fraxinea* in Finland and genetic variation among isolates sampled from Åland, mainland Finland, Estonia and Latvia. *Forest Pathology* 41: 169-174. doi: 10.1111/j.1439-0329.2010.00647.x.

Sander, H. 2000. Ülevaade Eduard Viiroki mõningatest töödest [An Overview of Some Works of Eduard Viirok]. Studies of woody plants in Estonia. Part V. Tallinn (in Estonian., abstr. in Engl.); ISSN 1406-2054;5.

Sander, H. 2001. Eesti viieteistkümne puittaimekollektsiooni ülevaade [Overview of Fifteen Collections of Woody Plants in Estonia]. Tallinn. 159 p. ISBN 9985816099.

Schoebel, C.N., Zoller, S., and Rigling, D. 2014. Detection and genetic characterisation of a novel mycovirus in *Hymenoscyphus fraxineus*, the causal agent of ash dieback. *Infection, Genetics and Evolution* 28: 78–86. doi:10.1016/j.meegid.2014.09.001.

Sibul, I. 2007. Saar on tamme noorem vend [Ash Tree Is a Younger Brother of an Oak]. Eesti Loodus, 58 (1), 6−11. ISSN 0131-5862. (In Estonian)

Tetlov, E., Bratka and M., Raudsaar, M. 2014. Reforestation. Yearbook Forest 2013. Estonian Environment Agency, 85-99.

The Forest Act. [https://www.riigiteataja.ee/en/eli/ee/Riigikogu/act/509022016005/consolide]

Thor, G., Johansson, P. and Jönsson, M.T. 2010. Lichen diversity and red-listed lichen species relationships with tree species and diameter in wooded meadows. *Biodiversity and Conservation* 19(8): 2307-2328. doi: 10.1007/s10531-010-9843-8.

Valk, U. and Eilart, J. 1974. Eesti Metsad [Estonian Forests]. Tallinn, Valgus, 308 p.

Zhao, Y.-J., Hosoya, T., Baral, H.-O., Hosaka, K. and Kakishima, M. 2013. *Hymenoscyphus pseudoalbidu*s, the correct name for *Lambertella albida* reported from Japan. Mycotaxon 122: 25–41.

Zheng, H.-D., Zhuang, W.-Y. 2014. *Hymenoscyphus albidoides* sp. nov. and *H. pseudoalbidus* from China. *Mycological Progress* 13**:** 625–638.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 125 – 139.

Ash dieback on the island of Ireland

A. R. McCracken[1,*], G. C. Douglas[2], C. Ryan[3], M. Destefanis[4], L. R. Cooke[5]

[1] Sustainable Agri-Food Sciences Division, Agri-Food & Biosciences Institute, 15A Newforge Lane, Belfast BT9 5PX, N. Ireland, UK, [2] Teagasc, Agriculture and Food Development Authority, Kinsealy Centre, Malahide Road, Dublin 17, Ireland, [3] Forest Service, Department of Agriculture, Food and the Marine, 3 West Agriculture House, Kildare Street, Dublin 2, Ireland, [4] Department of Agriculture, Food and the Marine, Pesticides, Plant Health and Seed Testing Laboratories, Backweston Campus, Celbridge, Co Kildare, Ireland, [5] School of Biological Sciences, Queen's University of Belfast, Medical Biology Building, Lisburn Road, Belfast BT9 7PL, Northern Ireland, UK

** corresponding author: a.mccracken@qub.ac.uk*

Abstract

On the island of Ireland it is estimated that there are over half a million kilometres of hedgerows (400,000+ km in the Republic of Ireland (Rep. Ireland) and 113,000+ in Northern Ireland (Northern Ireland). Ash (*Fraxinus excelsior*) is the second most important component, after hawthorn (*Crataegus monogyna*), in large proportions of this hedgerow network. In the Rep. Ireland over 20,000 ha of ash have been planted since 1990, primarily for sawlogs and to provide material for the manufacture of hurleys, which are used in an important national sport called hurling, and for camogie sticks used to play camogie. Ash dieback, caused by *Hymenoscyphus fraxineus*, was first identified on the island in October 2012 and since then has been detected at 306 sites (195 in Rep. Ireland and 111 in Northern Ireland). In the vast majority of cases the outbreaks have been on young, imported trees planted within the previous 5 – 6 years and it was evident that the pathogen had been introduced on trees for planting. On a small number of occasions there was evidence of the pathogen cycling within a plantation or moving from the plantation to infect neighbouring hedgerow trees. One possible mechanism by which the pathogen can build up sufficient inoculum is by the formation of apothecia on infected woody tissue high up on the plants. Rep. Ireland and Northern Ireland have strict policies of eradication and containment, as set out in the All-Ireland Chalara Control Strategy. To date over 2.1 million trees have been destroyed as part of an eradication strategy. It is considered that this prompt and far-reaching action has had a significant impact, significantly mitigating and preventing the rapid establishment of the pathogen and limiting its spread. The interventions since the disease was first confirmed have helped to protect the considerable investment in ash plantations of the last 20 years. The pathogen has not, however, been eradicated from the island of Ireland and it remains to be seen how widespread, and how quickly ash dieback will become established on the island of Ireland. The latest figures from the Republic of Ireland are that 733 hectares of ash plantation has been reconstituted with another species as a result of Chalara and this has cost our state €2.6 million so far; in addition, Chalara has been found and confirmed in all 26 counties of the Republic of Ireland to a greater or lesser extent. As a result the current policies and procedures regarding Chalara are under review.

Introduction

Ireland is an island 21 – 170 km distant from the west coast of another island, Great Britain, which in turn is separate from continental Europe. The prevailing winds tend to be south-westerly i.e. from the Atlantic Gulf Stream. These two factors mean that the probability of the natural spread of non-indigenous pests or pathogens into Ireland is significantly less than in most other parts of Europe. However, once introduced, the mild, moist weather conditions favour the growth and spread of many fungal pathogens. The relative isolation means that Ireland has had a high plant health status: it is currently free of many of the pests and pathogens which are currently causing significant damage to crops and trees elsewhere in Europe, but is still vulnerable. This is reflected in the 'Protected Zone' status for many organisms harmful to plant health that the Republic of Ireland and the United Kingdom (Northern Ireland) have under the EU Plant Health regime. Furthermore, experiences from continental Europe may not be directly applicable to Ireland. For example, many pathogens and pests found in Ireland have been introduced on plants or plant parts in trade rather than having spread from neighbouring infected regions. The maritime climate of cool summers and mild winters, high rainfall and little snow contrasts with much of northern continental Europe where extremes of temperature and rainfall are the norm. Hence any actions against pathogen or pest incursions to the island need to be considered in the Irish context.

While the island is divided into two EU Member State administrations: the Republic of Ireland (Rep. Ireland) and Northern Ireland (Northern Ireland) (a devolved administration of the United Kingdom of Great Britain and

Northern Ireland), the benefits of an harmonised all-island approach with regards to phytosanitary matters are recognised at both policy and delivery levels. There is, therefore, developing, ongoing close cooperation between the plant health services and scientists in both jurisdictions, i.e. the Department of Agriculture, Food and the Marine, DAFM (Rep. Ireland) and the Department of Agriculture and Rural Development, DARD (Northern Ireland).

In recent decades there has been a significant increase in the global trade of plants and plant products. It has been recognised that this movement of plants poses a high risk of introduction of unwanted organisms into areas which were previously free from them. It has been suggested that woodlands and native plant communities in the UK and Ireland are currently suffering from plant pathogens introduced, often on living plants, by human activity (Brasier 2008). This author suggested that the potential for future damage may be large and is critical of the international regulations aimed at mitigating the risks of introducing diseases, which he considers to be highly inadequate. High volumes of many categories of plants for planting in Rep. Ireland and Northern Ireland are grown outside Ireland, usually within the European Union (EU), presumably for reasons of supply availability and price competitiveness within the European Single Market (ESM). In contrast, there are limited imports of plants or plant parts from third countries outside the EU, and the phytosanitary regulations governing these tend to be very detailed and aimed at protecting the integrity of the ESM. As will be discussed presently it is highly probable that ash dieback, caused by *Hymenoscyphus fraxineus*, was introduced to the island of Ireland in ash trees for planting, brought from continental Europe which, in turn, was probably subject to intercontinental infection, initially from the far East (Drenkhan et al. 2014).

European or common ash (*Fraxinus excelsior* L.) is the only species of ash native to Ireland and is one of the commonest trees throughout the island, possibly because it has the qualities of a pioneering species that is well adapted to niche opportunities in a largely pastoral landscape, with very few old forests. It was one of the comparatively few tree species to colonise Ireland post glaciation and before Ireland became isolated from the European landmass. Ash has important heritage and ecological values in the landscape, both as a hedgerow and woodland species. In the Rep. Ireland ash is a significant component in 91% of native woodlands.

Rep. Ireland has an active afforestation programme and prior to the first finding of ash dieback, ash represented 10% of this afforestation, currently around 20,000 ha. In the period 1990 to 2009, an average of 670 ha of ash plantations were established per year. This represents a considerable investment and a growing resource, the value of which is increasing significantly as it moves towards maturity. The most active period of planting was in 2003 and 2004 when 1,800,000 plants were required each year from nurseries, a level of demand that necessitated imports of plants. Average plant demand was 1,300,000 yr^{-1} in the period 1997 to 2007. The overall areas of ash in various age classes are shown in Table 1.

Table 1 Areas (ha) of established ash in plantations and high forest in the Republic of Ireland.

Plantation type	Private plantation ash (grant aided)	Private forest (not grant aided)	Public forest (Coillte)	Total area
Period covered	*1980-2014*	*1980-2013*	*1800-2013*	
Area (ha)	15,995	8,318	4,015	28,328

Sources: Coillte – Coillte Sub-Compartment Database; Private Grant Aided – Department of Agriculture Afforestation Data; Private Non-Grant Aided – Ireland's National Forest Inventory 2012. Private Forest (not grant-aided) is comprised of both afforestation, reforestation and natural regeneration, i.e. predominately includes old estates and farms

Between 1990 and 2012 approximately 13,000 ha of ash were planted under Department of Agriculture, Food and the Marine (DAFM) grant support schemes. Ash is grown commercially for its dense, strong, but elastic timber, with targeted end use of timber for flooring and furniture and as sports goods (hurls) and wood fuel (firewood, smoking wood and barbeque charcoal). Traditional sports use ash in hurling and camogie, which trace their origins back to the Bronze Age and remain an important part of Gaelic heritage. In the sport of hurling, players use an ash stick (hurl or hurley) crafted from the butt of the tree where the grain runs into the root. There is an annual requirement for 360,000 hurleys, crafted from 2,000 m^3 of ash wood valued in the region of €450 per m^3 for logs at roadside. Currently 76% of this wood is imported. It had been hoped that by 2020, Ireland would be self sufficient in ash for hurleys when maturing plantations reach the required dimensions of 18 - 28 cm diameter at breast height (dbh) i.e. 1.3 m and this was seen as an important market for plantation thinnings. The fear is that if the disease becomes widespread this market will be lost and plantation logs will only fetch firewood prices of €60 per m^3. In response to the ash dieback outbreak, a Statutory Instrument required hurley makers to import only planks and debarked logs which significantly added to their production costs. This order has been rescinded recently as the risk of movement of the pathogen on wood and logs is deemed to be very small.

Ash is a vital part of the Irish landscape. There are numerous large mature ash trees all over the island. These may be remnants of former hedges or they may have been planted as parkland trees. However, the real landscape importance of ash is in Irish hedgerows. Hedges form the majority of field boundaries and act as stock-barriers. It may be that hedgerows could be a significant conduit for the spread of the pathogen. It had been estimated that there are in excess of 300,000 km of hedgerows in Ireland (Anon 2015a), which cover approximately 1.5 % of the land area. However in a further intensive study, The Heritage Council of Ireland (2015) estimated that hedgerows in Rep. Ireland have a linear dimension of 400,000 km. From GIS (Geographic Information System) and Lidar (Light Detection And Ranging) studies, the total area of hedgerows, individual trees, small woodland patches and scrub covers 350,000 to 450,000 ha in Rep. Ireland (Black et al. 2014; Green 2010; Sansford 2013). In many parts of the country ash forms a major component of those hedges. County surveys of hedgerows have shown ash is the second most frequent species after hawthorn (*Crataegus monogyna* Jacq.). The results of these surveys can be found at the website of The Heritage Council of Ireland (Anon. 2015c). The frequency of hedgerows which contain ash was lowest, at 10%, in Co. Offaly and highest, at 68%, in Co. Leitrim. Ash can also develop into trees and of the hedgerows surveyed, those which have ash as trees, ranged from 28% in Donegal to 75% in Longford.

In Northern Ireland there are fewer woodlands with an ash component but ash is still very important as a hedgerow tree, both in coppiced hedges and growing as single stem mature trees. It is estimated that there are 113,648 km of hedges in N. Ireland (McCann 2012) of which up to 90% have ash as a component. In some situations the ash is coppiced as part of the ongoing management of the hedges, while in other situations the ash trees are allowed to grow into mature single-stem, large trees. As in the Rep. Ireland, there is an active afforestation programme delivered by the Forest Service of the Department of Agriculture and Rural Development (DARD) and part financed by the EU. In Northern Ireland, however, ash is rarely the dominant species and is often included to add diversity to planting schemes.

Ash has great biodiversity significance. In a recent study of the importance of ash to UK biodiversity, Mitchell et al. (2014) identified a total of 1,058 species associated with ash trees. These included birds, mammals, bryophytes, fungi, vascular plants, invertebrates and lichens. Of these 44 were obligate, ash-associated species including fungi, invertebrates and lichens with a further 62 species including fungi, lichens, bryophytes and invertebrates highly associated with ash. Lawrence and Cheffings (2014) conclude that the contribution to UK biodiversity by ash trees is important and this is demonstrated by the dependence of 106 species on ash trees and the habitat that these trees create. Furthermore, of the 536 lichens that grow on ash in the UK, 220 are nationally rare or scarce, and 84 have been categorised as under threat (Mitchell et al. 2014). It can be assumed that the biodiversity associated with ash growing in Ireland will be very similar.

As a component of hedgerows and non-woodland patches, ash is important for carbon storage. In a study of Rep. Ireland hedges these two categories have been estimated to have a total carbon sequestration potential of 4.98 t CO_2 ha^{-1} yr^{-1} (Black et al. 2014). It is clear that the potential loss of a significant proportion of the ash population in Ireland to ash dieback would have large environmental, biodiversity, landscape and commercial impacts.

Although ash enjoys a widespread distribution in Ireland, most sites are sub-optimal for growth as a dominant forest tree and the dominance of ash in the Irish landscape is probably a comparatively recent phenomenon associated with field enclosures at the beginning of the 19th Century, as described for County Leitrim (Foulkes, 2006). In Europe its widespread success is also thought to be recent, reflecting its role in primary and secondary woodland succession, and is closely associated with forests dominated by European beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* (Matt.) Liebl.), pedunculate oak (*Quercus robur* L.), sycamore (*Acer pseudoplatanus* L.), black alder (*Alnus glutinosa* (L.) Gaertn.) or grey alder (*Alnus incana* (L.) Moench). Ash seems to become dominant where site conditions are less favourable for the associated species or where human manipulation of the environment occurs (Dobrowolska et al. 2011). Thus, the dominance of ash in Ireland may be due to lack of competition from other forest trees coupled with agricultural practices. Over time, if ash is lost from the hedgerows it would probably be replaced by other hedging species such as hazel. It is not clear if tree species such as sycamore would naturally become more common.

Ash dieback (*Hymenoscyphus fraxineus*)

Ash dieback is a fungal disease of *Fraxinus* spp. caused by the Ascomycete *H. fraxineus*, and the native ash species *F. excelsior* is particularly susceptible. The phenomenon of ash dieback has been observed on continental Europe, probably since the mid 1990s although the cause was not established until sometime later. The pathogen was first properly described in 2006 under the name *Chalara fraxinea* (Kowalski 2006). Four years later it was determined that *C. fraxinea* was the asexual (anamorphic) stage of the fungus when, in accordance with convention, it was subsequently named after the sexual stage *Hymenoscyphus pseudoalbidus.* The name by which it should now be known is *H. fraxineus* (Baral et al. 2014).

Ash leaves are infected in late summer by germinating ascospores of *H. fraxineus,* which grow into the petioles, the rachises, and then the shoot or stem where they initiate lesions. Infected petioles and rachises die and fall to the ground where they overwinter to produce apothecia which release ascospores the following summer (Gross et al. 2012). Once the pathogen is in the apothecia / ascospore phase of its life cycle, inoculum builds up quickly and initiates numerous fresh infections. Where ash dieback has been present for more than ten years in countries without effective control measures, a large proportion of ash trees have become infected and eventually succumbed to this pathogen (Timmermann et al. 2011).

All-Ireland Chalara Control Strategy

H. fraxineus is not a regulated disease under the EU Plant Health Directive (Council Directive 2000/29/EC). When the pathogen was first identified in Ireland in late 2012 the respective official Plant Health Services in the Rep. Ireland and Northern Ireland recognised that this was an issue affecting the entire island and that there were no natural or other effective barriers to air-borne spread of the fungal pathogen. Hence the DAFM in Rep. Ireland and the DARD in Northern Ireland developed a single All-Ireland Chalara Control Strategy (Anon. 2013). This strategy was described in an inter-Departmental cross-border document, which was launched in July 2013 by the Minister of State at DAFM, Tom Hayes TD (Teachta Dála), and the DARD Minister Michelle O'Neill MLA (Member of the Legislative Assembly).

The control strategy provided a framework for the policy of identification, control and eradication of *H. fraxineus* in Ireland and set out the actions required. The main aims of the strategy were described as:

- *All incidents of ash dieback disease found on the island of Ireland are managed consistently and promptly in order to contain and eradicate the disease and to minimise the risk of the disease spreading and becoming established.*
- *A programme of work and development for an evidence base in the context of the International Plant Protection Convention's International Standard for Phytosanitary Measure ISPM No. 4, in order to make the case for the establishment of a Pest Free Area by the end of 2013 and seek consideration by the EU Standing Committee on Plant Health to recognise pest free status in EU legislation, through designation as a Protected Zone.*

The first key objective was to 'Reduce the risk of the disease becoming established in the wider environment'. *'Wider Environment Infection'* is defined as "*ash of any age, which is thought to have been infected by the spread of spores in the environment. This is distinct from disease outbreaks in 'recently planted' or 'new planting' which is thought to have been introduced onto a site by planting previously infected forest nursery plants*" (Anon. 2013). Evidence from other European countries had shown that once the pathogen is established in the wider environment it is impossible to control or even restrict its spread (Timmermann 2011). It was clear initially that all of the sites in Ireland where ash dieback was confirmed were relatively recently planted (< 6 years), i.e. there was a very high probability that the pathogen had been introduced on infected plant material. Hence as a first step, emergency legislation was introduced to control the importation of ash trees, seeds and wood. In October 2012 both ministers announced a 'Fortress Ireland' approach and restricted any importation of ash trees to the island (Anon. 2012a) and made amendments to the Wood and Bark Order governing the importation of ash wood (Anon. 2012b). A policy of eradication was also adopted by plant health authorities north and south. DARD and DAFM also assisted the authorities in Great Britain in developing the necessary Pest Risk Analysis for Britain and Ireland (Sansford 2013).

In Northern Ireland, up until late 2012, ash was an important component of many new government grant-aided mixed woodland areas, similar to the schemes operated in the Rep. Ireland. At the first record of ash dieback in Ireland, as a temporary measure (still in force), both DARD and DAFM suspended grant-aid for new ash plantings in forestry schemes. If this temporary measure was going to be successful in protecting the Irish ash population then it was necessary to eradicate the pathogen and be able to source disease-free trees for any future planting. In 2013 The National Roads Authority, Rep. Ireland suspended the use of ash in roadside plantings and now uses alternative species. Other actions have included raising public awareness through information meetings ongoing information updates on the Departments' websites, press releases, and national and local adverts and the development by DARD and DAFM of the Tree Check App for reporting ill-health in trees. In May 2014 an all-Ireland conference on the disease was jointly organised by DAFM and DARD in conjunction with the Agri-Food and Biosciences Institute (AfBi), Northern Ireland and the Society of Irish Plant Pathologists. This conference was very well attended and brought together presentations and discussions on the latest scientific knowledge on the disease and input from stakeholders and other interested parties. Training and advice continues to be provided on the biology of the disease and recognition of symptoms and on the procedures around the clearance of affected forest plantations or infected nursery stock.

First records of ash dieback in Ireland

The first confirmed record of ash dieback on the island of Ireland was in October 2012 in a young forestry plantation in County Leitrim, Rep. Ireland, which had been planted in 2009 with trees imported from continental Europe. The trees on this site, and on all ten other sites which had been planted with the same batch of imported trees, were subsequently destroyed under official supervision. Since that finding there have been a total of 195 confirmed findings in the Rep. Ireland (Table 2).

Table 2 Confirmed findings (as of 12th November 2015) of ash dieback disease (*Hymenoscyphus fraxineus*) in the Republic of Ireland (DAFM, 2015).

Location type	Confirmed findings Oct 2012 – Dec 2014	New findings in 2015 (12th Nov 2015)	Total (12 Nov. 2015)
Forestry plantations[1]	54	56	110
Horticultural nurseries	20	5	25
Garden Centres	4	0	4
Private gardens	7	0	7
Farm / Agri-environment plantings	23	2	25
Roadside plantings[2]	4	9	13
Hedgerows[3]	4	7	11
Total	116	79	195

[1] Refers to the total number of forestry plantations with confirmed findings.

[2] Refers to the number of counties with one or more confirmed findings in roadside plantings.

[3] Refers to the number of counties with one or more confirmed native hedgerow findings

The findings set out in Table 2 demonstrate the variety of settings in which infected ash trees have been found in the course of surveys conducted by DAFM inspectors. The vast majority of infected sites have now been cleared of ash plants. DAFM introduced a Reconstitution Scheme grant to support forest owners affected by the disease in the removal and destruction of ash trees and leaf litter and the reconstitution of the forest with alternative species to ash. To date, approximately 2 million ash trees have been removed and destroyed by deep burial or burning, with particular attention paid to the management of leaf litter because of its important role in the disease cycle. Where achievable, trees in an infected site were removed before flushing to reduce inoculum development and as far as possible material already on the ground was collected although it was recognised that it was very difficult to remove all potentially infective plant parts.

The most significant sites in terms of the epidemiology of the disease are those in the 'hedgerow' category. While the findings in all the other categories were clearly linked to imported planting material, incidence of the disease in hedgerows suggests probable spread of the disease in Rep. Ireland. In Northern Ireland the first finding of ash dieback was on plants in a recently established plantation in November 2012. Since then there have been 111 sites in which the pathogen has been confirmed (Table 3).

In almost every case the plantations affected were recently planted (< 6 years), and it was often possible to trace back to the original sources of the planting material. The sources were a limited number of nurseries outside Ireland and routinely the origins of the plants were on continental Europe. Having identified the source it was possible, with excellent co-operation from the nurseries involved, to trace forward to plantations established using plants from the same or similar batches of young trees. This intensive trace-backward / trace-forward surveillance led to identification of many new infected sites which were found early in 2013 and were destroyed soon thereafter.

Table 3 Confirmed findings (November 2015) of ash dieback disease (*Hymenoscyphus fraxineus*) in Northern Ireland (DARD, 2015).

Site Type	Age	Number of Outbreaks 2012/13 (Premises)	Number of Outbreaks 2014 (Premises)	Number of Outbreaks 2015 (Premises)	Total
Woodland Plantation	0-10	63	1	17	81
Urban/Amenity	0-5	9	0	1	10
Roadside	0-5	3	0	0	3
Private Garden	0-5	10	0	0	10
Hedgerow (recently planted)	0-5	3	1	0	4
Nursery Findings	0-5	3	0	0	3
Total		91	2	18	111

Systematic and targeted surveys

Following detection of the disease, plant health inspectors carried out intensive surveillance programmes. The initial approach to surveillance was one of ‘trace-back / trace-forward’. Traceability mechanisms which are required under Council Directive 1999/105/EC on the marketing of forest reproductive material have enabled good ‘trace forward’ of imported forestry planting material, although similar traceability is not available in non-forestry sectors such as roadway plantings. Further to this, there were both structured surveys, i.e. to get maximum coverage of the whole country, and targeted buffer surveys, i.e. associated with outbreaks, which had been confirmed through laboratory tests.

DAFM has carried out intensive surveys for the disease since the first finding in 2012. The surveys have been carried out in line with ISPM No. 6 (Anon. 2005) incorporating systematic and targeted, risk-based components. Targeted forestry surveys have involved inspections of hundreds of plantations where imported ash plants had been planted. Where infected ash plantations have been cleared, follow up surveys have been carried out in subsequent years to identify any evidence of spread from the original infected sites to the surrounding environment.

In the Rep. Ireland the systematic survey element was based on a 2 x 2 km grid. Approximately 370 sites (both National Forest Inventory plots and hedgerow points) were surveyed. To date the results of the systematic grid-based survey have been negative. In addition to these elements, DAFM follows up on reports of suspicious ill-health of trees from stakeholders.

In both the Rep. Ireland and Northern Ireland many of the new plantations that have been planted in the recent past have been supported by Government planting schemes. Using this information, further plantations were identified which were surveyed and on some occasions were found to be infected. It was more difficult to identify where ash had been planted as part of a landscaping programme, e.g. new roadsides, as these were usually not grant-aided and therefore details of sources of plants were not always available. Such sites were often only identified by inspectors on an *ad hoc* basis.

In Northern Ireland, at every site where ash dieback was identified, a standard 500 m buffer survey was carried out. At sites where there was evidence of spread within or without the plantation an intensive survey was conducted on all hedgerows including both roadside hedges and field boundaries within a 250 m radius. Any plants with dieback symptoms were submitted to the laboratory for diagnosis. A less intensive survey was conducted out to a distance of 500 m. In 2015 (until November) alone, in total, DARD inspectors conducted over 1,800 surveys (Figure 1A).

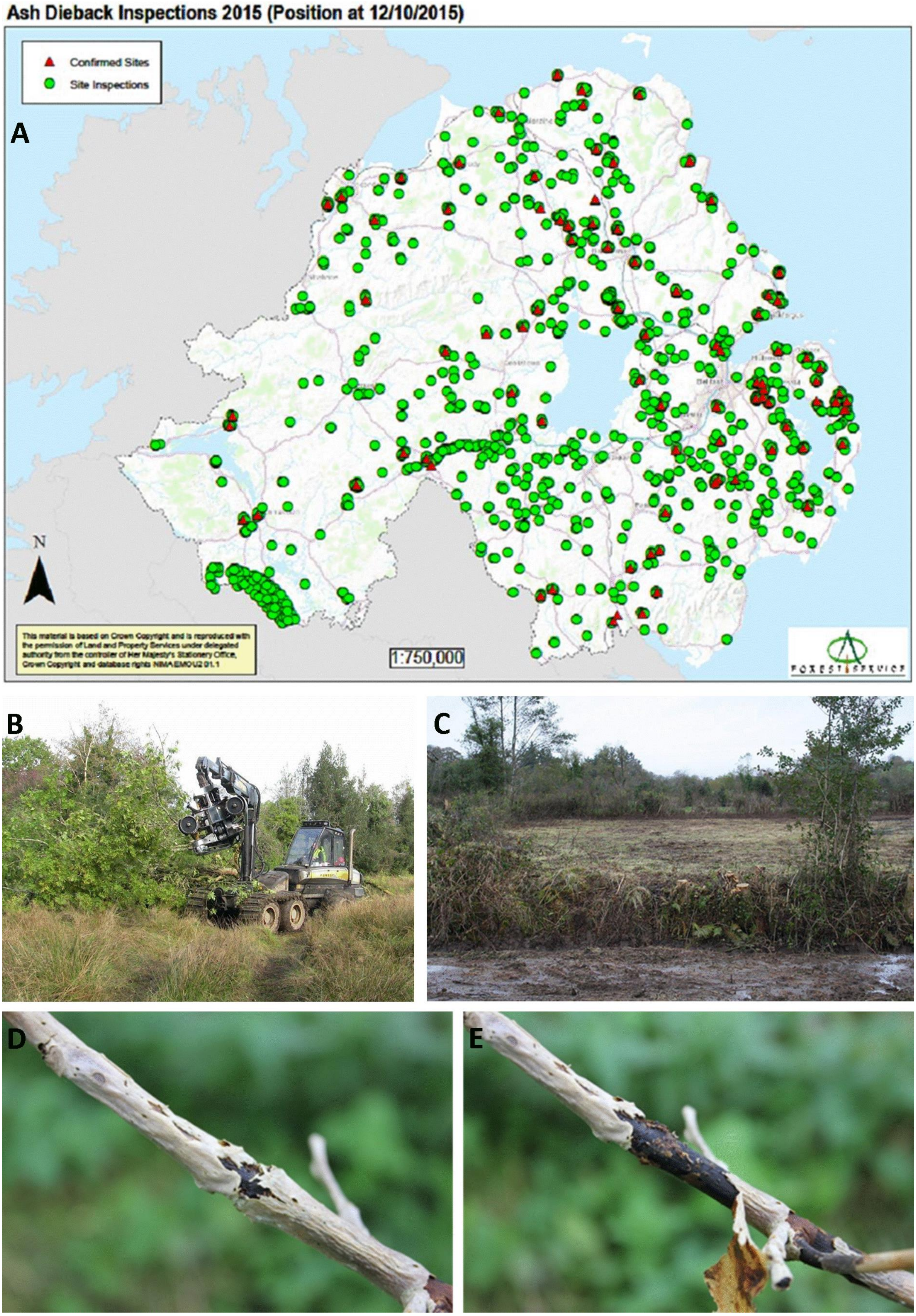


Figure 1 (see next page for descriptions)

Figure 1A) Northern Ireland plant health inspections of ash plantings where ash dieback could be present (these include buffer surveys of areas associated with previous outbreaks; trace-forward / trace-back plantings; *ad hoc* findings; structured surveys) carried out January – November 2015. Green dots were survey sites where ash dieback was not detected. Red triangles were sites where symptoms of ash dieback were observed and the pathogen *H. fraxineus* was detected using laboratory tests. **1B)** Harvester at work at the Leitrim site (Coillte, DAFM). **1C)** Cleared field post eradication works (Forest Service, DAFM). **1D-1E)**Woody tissue still attached to dead tree with papery epidermis and remains of apothecia below the epidermis.

On the confirmation of the presence of the pathogen on a site a Statutory Plant Health Notice was issued preventing the movement of plants from the site, imposing bio-security measures and prohibiting further planting of ash at the site and requiring the removal and destruction of all ash plants and associated plant debris.

Detection of *Hymenoscyphus fraxineus*

Inspectors collected samples of ash trees with dieback symptoms. Great care was taken to avoid cross-contamination between samples because of the sensitivity of the detection methods, particularly those based on DNA analysis. Inspectors wore fresh disposable nitrile gloves for every sample, equipment was flame-sterilised between samples and samples were bagged separately, along with the gloves used, and sealed. Before leaving a site inspectors used a biocide to spray boots and clothes to avoid potential movement of the pathogen to disease-free sites. Samples were submitted to either the Agri-Food & Biosciences Institute Laboratories in Belfast or to the DAFM Laboratories at Backweston, Co. Kildare. Routinely, DNA was extracted from samples and subjected to validated Real-Time PCR testing using *H. fraxineus* specific probes and primers (Ioos et al. 2009; Ioos and Fourrier 2011). This enabled large numbers of samples to be processed rapidly and results were very consistent. This methodology is highly sensitive giving C_t (cycle threshold) values in the range 18 – 32. On occasions where low-level positives (C_t values > 32) were recorded a fresh sample was collected and sent to the laboratory for analysis.

Routinely, but not with every sample submitted, an attempt was made to isolate the pathogen. The best success was obtained when isolating from fresh, large, well-developed stem lesions, onto Potato Dextrose Agar (PDA) plates. After placing the piece of plant tissue on the agar, the plates were incubated for up to four weeks at 4° C before transferring them to 18° C after some growth of *H. fraxineus* had developed. Isolation was obviously of limited value for routine processing of samples but did enable laboratories to build up a culture collection of isolates obtained from trees growing in Ireland. It was also useful in confirming that the pathogen was viable and growing actively.

When ash dieback was confirmed at a site, a search was made for infected leaf rachises and petioles with or without apothecia. This was to indicate whether or not the pathogen had entered the epidemic phase of the life cycle. In all but the most heavily affected sites, few apothecia were found in the field. On several occasions apothecia did develop after a few weeks' incubation of plant material under high humidity in the laboratory at room temperature in the light. Apothecia were subjected to molecular analysis to confirm that they were *H. fraxineus* (*pseudoalbidus*) and not *H. albidus.* The latter was not detected in any routine samples but was confirmed by AFBI analyses in samples collected by DAFM in samples from Rep , Ireland, counties Cavan (May 2015) and Leitrim (June 2015).

Furthermore, *H. albidus* was found as ascocarps on ash petioles in the leaf litter of a 10-year-old gene bank of ash trees in Dublin in September 2015. It was notable that the fructifying rachises were found in the first and second row of trees near the edge of the plantation. Typical asci and spores were observed using light microscopy (Figure 2) and the absence of a characteristic crozier at the base of the asci was noted. The crozier feature is a characteristic feature *H. pseudoalbidus* (*fraxineus*) (Zhao et al. 2012). DNA was extracted from the ascocarp tissue and was confirmed as *H. albidus* by real- time PCR using the TaqMan method capable of specifically detecting *H. albidus* described in Husson et al. 2011. These were the first recorded confirmation of *H. albidus* in Ireland.

Eradication

In Northern Ireland when *H. fraxineus* was detected on a site all of the ash trees within that plantation were destroyed. If the trees were very small it was usually possible to uproot them. Larger trees were cut as close to ground level as possible. The cut stump was treated with glyphosate herbicide to prevent re-growth. These sites were checked the following year to ensure that all trees had been killed. The cut trees were deep buried, burned *in situ*, or removed to a landfill site. On bigger plantations and with larger trees they were chipped, under cover, into a covered trailer and the chips put in a landfill site or burned *in situ*. The cost of removal was borne by the Government Forest Service and the landowners were offered freely a selection of tree species to re-establish their woodlands. As far as was practically possible, great care was taken to remove as much as possible of the plant debris on the ground. If trees could be removed before autumn leaf fall this was considered highly desirable. A similar protocol was followed in the Rep. Ireland.

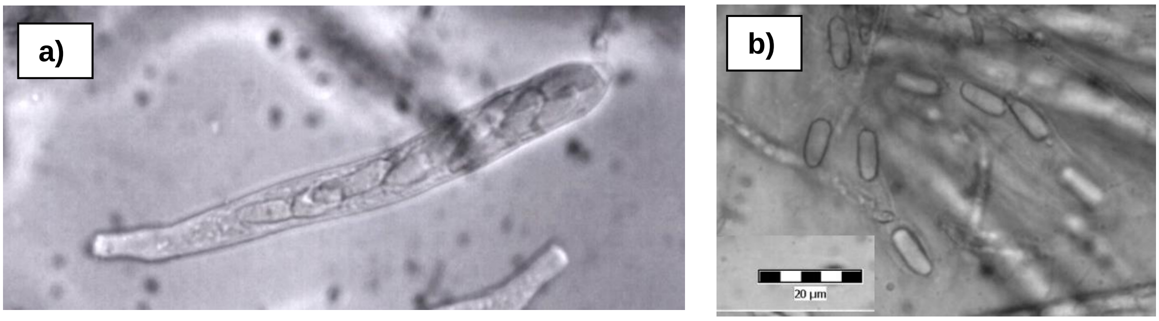


Figure 2 a) Ascus and **b)** spores of *H. albidus* from an Irish source

Case study 1: Co. Leitrim, Republic of Ireland

As mentioned previously, the first case of ash dieback in Ireland was confirmed in October 2012 in a young forestry plantation in County Leitrim (N: 54° 05'; W: 7° 48'), which had been established in 2009 with *F. excelsior* trees imported from continental Europe. The Leitrim forest plantation was 3.85 ha of which 2.3 ha was ash. All planted ash trees (5,700) at the site were cut, the stumps treated with herbicide and the cut trees and leaf litter burnt on site in October 2012. At the time of clearance, the trees were c. 2 m in height and approximately 50% of the planted ash showed symptoms of the disease. Ash was plentiful in internal hedgerows on the site and in the vicinity of the site but at this stage symptoms were not observed in the hedgerows.

Surveys in the 2013 growing season, however, led to confirmation of the presence of the disease in a number of hedgerow trees within and beside the original infected plantation site. At the time this was the first site on the island where the evidence suggested spread of the disease from imported material to wild ash trees. Following demarcation surveys, a major eradication effort was then undertaken at this site commencing in October 2013 with the aim of removing all host material and leaf litter. A 250 m buffer zone was demarcated around the plantation, which incorporated all the known positive trees. This resulted in a 60 ha demarcated zone and contained approximately 12 km of hedgerows of which 7.5 km contained ash. All host material within the demarcated area was removed and destroyed. Tree clearance involved a commercial timber harvester (Figure 1B), a forwarder and a team of three chainsaw operators.

Stumps of cut trees were treated with herbicide. An excavator was used to gather branch material and leaf litter, including clearing drains of material, for burning on site (Figure 1C). In total 850 ash trees >10 cm dbh and 850 ash trees <10 cm dbh were felled and destroyed.

In early 2014 further clearance operations were carried out to eliminate any material that was missed and any re-growth within the demarcated zone was cut and sprayed with herbicide. Follow- up surveys were carried out in the wider area. This led to the detection of a number of infected trees outside the demarcated area up to approximately 2 km from the original infected site. In 2015, surveys led to further findings of the disease at increased distance (over 10 km) from the original site.

Case study 2: Co. Antrim, Northern Ireland

In August 2015 in Co. Antrim a small mixed plantation including ash was found to have a high level of *H. fraxineus* infection. The trees had been planted in 2008 using imported ash saplings from a nursery in Great Britain. On initial survey of the site a high proportion of trees was displaying some level of infection. Symptoms of ash dieback on infected trees were classified into broad groups in order to postulate a disease time-line:

- Infected at planting: These trees were small and many had died within the first one to three years of planting. Often the leaders had died and there was evidence of shoot production from the base of the plants.
- Side shoot infections: Side shoots and small branches were dead due to infections which had occurred through leaves and petioles. By assessing the extent and size of the shoots and associated lesions it was possible to estimate the year that leaf infection have occurred.

- Basal lesions: Large lesions occurring at the base of the trees at soil level. These lesions were not associated with any stem infections higher up. It was postulated that rachises and petioles trapped in grass had produced large numbers of apothecia and copious amounts of ascospores which infected the base of the trees. Lesions near the base of the trees could have been the result of mechanical damage during uprooting in the nursery. Several of these lesions were confirmed by molecular methods to be infected with *H. fraxineus*.

- Stem lesions: Characteristic diamond-shaped lesions which appeared to have developed from a side shoot infection rather than on a plant which had been infected prior to planting.

Leaf symptoms, spotting, vein and petiole lesions and wilting, which had occurred during the growing season 2015 apparently emanating from apothecia formed as a result of 2014 leaf infections and / or from other airborne incursions.

It was clearly evident that the pathogen had been, and still was, actively circulating within the plantation. This raised the question as to how inoculum had built up sufficiently. When planted, the trees were leafless, two-year-old saplings with no obvious symptoms, and yet infected rachises were found producing sporulating apothecia on the plantation floor. It was considered unlikely that the pathogen had grown from the stem lesion back into the rachises or petioles of uninfected shoots.

Initial development of apothecia

In the life cycle proposed by Gross et al. (2012) apothecial development occurs on fallen petioles and rachises with ascospore release during the summer months. For this to occur it is necessary to have had infection initially on the leaves. Once this occurs, then the life cycle can continue and the inoculum can build up exponentially. However, the trees, which were planted in Ireland and subsequently developed ash dieback symptoms on the stems at all the sites, had been imported as small (generally two-year-old) leafless saplings. It can be assumed that symptoms were not obvious at the time of planting, since such plants would have been rejected. Since no leaves were present on the saplings there would have been no petioles or rachises on which apothecia could form as described by Gross et al. (2012). This therefore raised the question about how the pathogen moved from a lesion on the stem to colonise petioles and rachises, which in turn can develop apothecia.

A number of hypotheses were considered.

1. Introduction of the pathogen on leaf and rachises debris

When plants are bundled in the nursery for transportation for planting it is inevitable that there is a certain amount of plant debris and leaves associated with them. If the plants were coming from an infected nursery it may not be surprising that some pathogen inoculum would be introduced in this way. Infected rachices may harbour the pseudosclerotial stage of Chalara, for one or more years before optimal conditions prevail to favour the onset of ascospore production and so they have the potential to become inoculum sources over time. It is relatively unlikely that a nursery would be heavily infected and continue commercial production of ash plantssince, apart from other considerations, this would be uneconomic, therefore only low levels of inoculum would be introduced by this route and this would take a considerable time to initiate significant amounts of infection.

2. Movement of the pathogen out of the stem lesion back along a petiole to cause a leaf infection.

This was thought to be unlikely and no evidence was ever observed of the pathogen moving from the stem into the petiole.

3. Development of apothecia on roots.

This was considered not impossible and the likelihood of it occurring has been discussed during FRAXBACK meetings. At the Co. Antrim site, on the roots of many of the dead young trees which were examined, pseudosclerotia were observed and on a small number of these apothecial formation was induced following incubation in the laboratory. The development of apothecia on infected roots has been thought to be a very rare occurrence and this is the first report for Ireland. Nevertheless the experience of this site would suggest that under suitable conditions apothecia can form on roots readily. They seemed to occur more commonly on roots from trees which had been growing in water-soaked soil.

4. Development of apothecia on woody tissue

Several trees on the site had been dead for some time. The epidermis on their shoots and small branches had become pale and papery in appearance. This was easily peeled away and on several occasions fully developed apothecia were found growing on the wood (Figure 1D and 1E). This occurred at a height of up to 3 m on many trees. While ascospores were unlikely to escape from beneath the dried epidermis while the branches were *in situ,* when these branches fell to the ground, apothecia could be exposed and release their spores. The spread of the pathogen within the plantation was relatively rapid at sites where there was a significant initial level of tree death. If large numbers of trees were dying in the first year or two after planting then the potential for significant inoculum to build up on the woody tissues was high. This is therefore considered to be possibly an important source of inoculum development.

The potential development of apothecia on shoots and roots is of significantly greater importance in Ireland than other countries where aerial inoculum is the primary means of dissemination. The Irish maritime climate and in particular wet soils may also influence the formation of apothecia on these plant tissues. The period of ascospore production would appear to be extended into the autumn and the lack of severe winter temperatures could encourage pathogen survival.

Spore sampling

Four Burkard® spore samplers were employed at a range of sites across Northern Ireland in 2014 and 2015. In both years between May and October a sampler was sited at Glenwherry Hill Farm (NW 397 567). The Hill Farm is on the Antrim Plateau in the east of Northern Ireland, where there were almost no ash plants in the landscape. However, the mathematical models developed by the University of Cambridge, incorporating data supplied by DAFM and DARD, indicated that if *H. fraxineus* ascospores were airborne, particularly if originating from Great Britain or the continent, this would be the most likely area in which they would be deposited (Matt Castle; personal communication). No ascospores were detected. In 2014 two samplers were placed in a positive ash dieback site in Co. Tyrone (NV 564 142) from which all ash trees had been removed the previous winter. No ascospores were detected. In 2015 a sampler was run from May to October at a positive site in Co. Londonderry (NW 101 702). At this site, infected plants had been removed in 2013, but a small clump of larger trees had been missed and was not removed until late 2014. There was some evidence of apothecial development on fallen rachises. By mid September small numbers of spores were observed and in one week only in late September, coinciding with a period of warm dry weather, there was significant release of spores. Thereafter, only small numbers of spores were observed. A sampler was run from July to November in the far west of Northern Ireland in Co. Fermanagh (NV 213 003) close to the border with the Rep. Ireland and approximately 20 km from the site described in Case Study 1. While the running of the sampler was somewhat inconsistent, no ascospores were detected during the summer. The fourth sampler was placed at the Co. Antrim site, Case Study 2 (NW 206 518) in mid August. Throughout the summer infected rachises were commonly found. Very few spores were detected during August. However in mid September there was evidence of profuse spore production similar to that found at the Co. Londonderry site. While this reduced significantly after that, spores were still being observed up until early November.

In summer 2015, DAFM conducted a sporetrapping experiment around two infected sites in collaboration with and using a methodology developed by INRA, France’s National Institute for Agricultural Research. The experiment aimed to detect, quantify, and establish dispersal patterns around these known positive locations. Results are still being analysed.

Potential to identify ash dieback resistant ash

The genetic diversity of Irish ash populations was analysed using neutral markers and this has shown that 96% of the variation is contained within the population with little evidence of population structuring (Thomasset 2011; Thomasset et al. 2013). This apparent lack of genetic diversity compares with results from other countries (Heuertz et al. 2001; Morand et al. 2002; Fernández-Manjarrés et al. 2006; Ferrazzini et al. 2007). This means that it will be unlikely to identify distinct populations of dieback resistant trees, since the probability of sampling differentiated individuals within a population is similar to the probability of sampling differentiated individuals in two different populations, because of an almost random distribution of genetic diversity within and among populations. Molecular markers were also used in efforts to characterise hybrids of ash which were imported to Ireland in the period 1990-2002. The material was shown to consist of hybrids of *F. excelsior* with *F. angustifolia*. In many cases, imported trees were mal-adapted showing poor growth and stem form in plantations (Douglas and Thomasset 2014; Thomasset et al 2011b). The percentage of hybrid trees detected in two intensively sampled plantations ranged from 28 - 58%. These imports were referred to as cryptic hybrids because of a lack of intermediate morphological features which are common in pure F1 hybrids (Thomasset et al. 2013; Thomasset et al 2011a). Some hybrid trees still remain after thinning, however their susceptibility to *H. fraxineus* is not known. Efforts to find Irish genotypes of ash with tolerance to *H. fraxineus* are underway by Teagasc, the Rep. Ireland agricultural science research organisation. Irish genotypes of ash were propagated vegetatively in 2013 and planted out in test environments with high disease pressure in 2014 (France and Lithuania). At the end of the first season of growth, evidence for infection had already been found among the trees planted in Lithuania indicating good prospects for finding *H. fraxineus* tolerant genotypes by this approach.

DAFM is also participating in a five-year project which was begun in 2013, the aim of which has been to produce individual trees of ash which show resistance or tolerance to Ash Dieback and to use them to bulk up stocks of resistant trees as well as to establish seed-producing orchards with resistant parent trees. The project, which is part funded by DAFM, is being carried out by Forest Research, an agency of the Forestry Commission in the UK. The project involves 48 hectares of trial plantings over fourteen sites in the east of England and the mass screening of some 155,000 ash trees with fifteen different provenances from continental Europe, the UK and Ireland. Over 14,000 Irish ash plants from two distinct seed lots are included in the trials.

Conclusions

Protection of the plant health status of Ireland is a high priority. It is clear that ash dieback was brought into the island of Ireland on infected plants for planting. This emphasises that growers have a high degree of responsibility towards their customers to produce, and trade in disease-free plants. Similarly, landowners should become more fully aware of the materials they deploy in forests and gardens: i.e. the provenance of the seeds / saplings, the locations of sapling production and the traceability systems in operation. Local nurseries and producers should take up opportunities to grow native plants for local usage and customers should be prepared to meet cost differentials occasioned by, for example, lack of economies of scale in local nurseries.

There may be an argument that Government could help with facilitating more sustained local sapling production by forward planning of grant-aided forestry Schemes, since it is in its long-term interest. Sustained forward planning is also in the interest of nurseries because they require at least three years notice to supply plants for any new grant scheme which will potentially increase the demand for trees. To date the interventions undertaken by the respective Government Departments have had a major impact in mitigating the establishment of ash dieback in Ireland and in attenuating its spread to the wider environment. *H. fraxineus* is, however, a highly infective pathogen and it is therefore very difficult to predict if it will be possible to prevent the further spread of ash dieback within Ireland.

There is reasonable confidence that no new inoculum of *H. fraxineus* has been introduced into the island through trade since the ban on importation of ash plants for planting, although there is the risk of some importation occurring through unregulated trade. The policy restricting the movement of ash plants into Ireland may have to change in the near future, in line with general EU legislation, which will potentially increase the risk of future introductions. Current epidemiological modelling has suggested that ingress of ascospores from Great Britain or Europe in numbers sufficient to incite an epidemic is unlikely. This situation may change as the sources and quantity of inoculum production increases throughout the UK and Europe. Despite findings of the disease in a total of over 300 sites or holdings on the island of Ireland, these have consisted almost exclusively of material planted within the last six years. However, there are a few places on the whole island where it could be considered that the pathogen has spread to trees in the hedgerow environment.

In both the Rep. Ireland and Northern Ireland there have been a few locations where the pathogen has been detected in isolated trees or hedges with no obvious link to infected sites. These anomalous cases may be explained by ingress of aerial spores and / or by spore production from infected trees from unknown sources in nearby landscape plantings. However the findings of infections in hedgerows associated with diseased plantations illustrate the highly infective nature of the pathogen, These localised infections probably originated from ascospores produced on small quantities of leaf debris and / or necrotic shoots and roots. While it is known that the pathogen can spread very rapidly across regions where there is an adequate aerial inoculum, the authors consider that the current situation in Ireland is of a slow rate of spread (based on survey data). Continued intensive surveying and monitoring of positive sites, together with data from spore trapping, will provide evidence for the likelihood of success of the current disease eradication policy. Removal and destruction of infected ash trees and leaf debris at infected plantations will contribute to reducing the build up of inoculum and slowing the spread of the disease.

There are over 800 risk entries on the Department of Environment, Food and Rural Affairs (DEFRA) Plant Health Risk Register (Anon. 2015b). Many of these are potential high plant health risks to agricultural, horticultural, forestry and amenity crops and plants in Ireland. It is therefore essential to engage in horizon-scanning in order to identify threats at an early stage. Furthermore, because of the constant issue of emerging diseases and pests, a more rigorous system of traceability is now advised for all categories of imported trees and shrubs throughout Europe. Consideration also needs to be given as to how pests and pathogens can more effectively be detected at points of importation.

Ash is a high profile species. The disease has led to increased awareness among the public and industry of the importance of plant health and the potential negative implications of trade and non-trade/other transfers of plant material on plant health status. This provides an opportunity to develop greater future public engagement with plant health, e.g. by using the Tree Check app for wider plant health monitoring. The latest figures from the Republic of Ireland are that 733 hectares of ash plantation has been reconstituted with another species as a result of Chalara and this has cost our state €2.6 million so far; in addition, Chalara has been found and confirmed in all 26 counties of the Republic of Ireland to a greater or lesser extent. As a result the current policies and procedures regarding Chalara are under review.

Acknowledgements

The authors acknowledge the support and information supplied by the Forest Service DAFM, the Horticulture and Plant Health Division, DAFM, the Plant Health Laboratory, DAFM and the Plant Health Inspectorate, Forest Service, DARD. We also acknowledge the close working relationships between Plant Health Policy colleagues in DARD and DAFM and the support and input from operations experts. The opportunity to be part of the EC COST Action FP1103 FRAXBACK network is greatly appreciated. Alistair McCracken acknowledges the hard work of Lisa Quinn, Mark Wilson, David Craig, Sean Lennon (AFBI) and Emma Baxter (QUB) in collecting data and samples, managing the spore samplers, examining the tapes and in the preparation of this manuscript. The rapid processing of large numbers of suspect ash samples from both Northern Ireland and Rep. Ireland by the molecular plant diagnostics laboratory of the Agri-Food & Biosciences Institute is gratefully acknowledged.

References

Anon. 2005. International Standards for phytosanitary measures; ISPM No. 6. Guidelines for Surveillance (1997). Secretariat of the International Plant Protection Convention (FAO, 2005). http://www.acfs.go.th/sps/downloads/13717_ISPM_6_E.pdf

Anon. 2012a. Fortress Ireland approach to prevent spread of ash disease essential. http://www.northernireland.gov.uk/index/media-centre/news-departments/news-dard/news-dard-october-2012/news-dard-261012-fortress-ireland-approach.htm (accessed 23rd November 2015).

Anon. 2012b. Plant Health (Wood and Bark) (Amendment) Order (Northern Ireland) 2012. http://www.legislation.gov.uk/nisr/2012/400/article/2/made (accessed 23rd November 2015).

Anon. 2013. All-Ireland Chalara Control Strategy. https://www.dardni.gov.uk/publications/all-ireland-chalara-control-strategy (accessed 23rd November 2015).

Anon. 2015a. Hedgerows. Notice Nature Ireland http://www.noticenature.ie/files/hedgerows.pdf (accessed 19th October 2015).

Anon. 2015b. UK Plant Health Risk Register: Department for Environment, Food & Rural Affairs. https://secure.fera.defra.gov.uk/phiw/riskRegister/ (accessed 10 December 2015).

Anon. 2015c. Heritage Council of Ireland 2015. New Initiatives Launched to Safeguard Ireland's Unique Hedgerows - accessed 20 November 2015). County surveys: See *http://www.heritagecouncil.ie/* (accessed 20th November 2015).

Baral, H-O, Queloz, V and Hosoya, T. 2014. *Hymenoscyphus fraxineus*, the correct scientific name for the fungus causing ash dieback in Europe. *IMA Fungus* 5: 79 - 80

Black, K., Green, S., Mullooly, G. and Povida, A., 2014. Towards a national hedgerow biomass inventory for the LULUCF sector using LiDAR remote sensing (2010-CCRP-DS-1.1): Final report, EPA, Dublin. https://www.epa.ie/pubs/reports/research/climate/ccrp-32-for-webFINAL.pdf

Brasier, C. M. 2008. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* 57: 792 – 808.

DAFM, 2015. Ash dieback (Chalara) http://www.agriculture.gov.ie/forestservice/ashdiebackchalara/ (accessed 20th November 2015).

Drenkhan, R., Sander, H. and Hanso, M. 2014. Introduction of Mandshurian ash (Fraxinus mandshurica Rupr.) to Estonia: Is it related to the current epidemic on European ash (F-excelsior L.)? *European Journal of Forest Research* 133: 769 – 781.

Dobrowolska, D., Hein, S., Ossterbaan, A., Wagner, S., Clark, J. and Skovsgaard, JP. 2011. A review of European ash (*Fraxinus excelsior* L.) implications for silviculture. *Forestry* 84: 133 – 148.

Douglas, G. C. and Thomasset M. 2014, Identifying and characterising hybrid 'brown bud' ash in Ireland. *COFORD Connects- Reproductive material* No. 20, 1-6.

Fernández-Manjarrés, J. F., Gerard, P. R., Dufour, J., Raquin, C. and Franscaria-Lacoste, N. 2006. Differential patterns of morphological and molecular hybridization between *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl (Oleaceae) in eastern and western France. *Molecular Ecology* 15: 3245 – 3257.

Ferrazzini, D., Monteleone, I. and Belletti, P. 2007. Genetic variability and divergence among Italian populations of common ash (Fraxinus excelsior L.). *Annals of Forest Science* 64: 159 – 168.

Foulkes, N. .2006. County Leitrim hedgerow survey report. *An Chomhairle Oidhreachta / The Heritage Council* 2006. pp 92

Green, S., 2010 The Irish hedge map – version 1.0 Teagasc Technology Update www.teagasc.ie (accessed 20th November 2015).

Gross, A., Zaffarano, P. L., Duo, A. And Grunig, C. R. 2012. Reproductive mode and life cycle of the ash dieback pathogen. *Fungal Genetics and Biology* 49: 977–986.

Heurtz, M., Hausman, J-F., Tsvetkov, I., Franscaria-Lacoste, N. and Vekemans, X. 2001. Assessment of genetic structure within and among Bulgarian populations of the common ash (*Fraxinus excelsior* L.). *Molecular Ecology* 10: 1615 – 1623.

Husson, C., Scala, B., Cael, O., Frey, P., Feau, N., Ioos, R. and Marcais, B. 2011 *Chalara fraxinea* is an invasive pathogen in France. *European Journal of Plant Pathology*,130 (3): 311-324.

Lawrence, R. and Cheffings, C.M. 2014. A summary of the impacts of ash dieback on UK biodiversity, including the potential for long-term monitoring and further research on management scenarios. *Joint Nature Conservation Committee, Report No. 501.* Peterborough 2014.

Ioos, R. and Fourrier, C. 2011. Validation and accreditation of a duplex real-time PCR test for reliable *in planta* detection of *Chalara fraxinea*. *OEPP/EPPO Bulletin* 41: 21 – 26.

Ioos, R., Kowalski, T., Husson, C. and Holdenrieder, O. 2009. Rapid *in planta* detection of *Chalara fraxinea* by a real-time PCR assay using a dual-labelled probe. . *European Journal of Plant Pathology* 125: 329 – 335.

Kowlaski. T. 2006. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxineus excelsior*) in Poland. *Forest Pathology* 36: 264 – 270.

McCann, T. 2012. The Woody Species Diversity of Hedges in Relation to Environment, Landscape, History, Management and Structure in Northern Ireland. PhD Thesis, Queen's University of Belfast 2012.

Mitchell, R.J., Bailey, S., Beaton, J.K., Bellamy, P.E., Brooker, R.W., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Farren, J., Gimona, A., Goldberg, E., Hall, J., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Hooper, R.J., Howe, L., Iason, G.R., Kerr, G., Littlewood, N.A., Morgan, V., Newey, S., Potts, J.M., Pozsgai, G., Ray, D., Sim, D.A., Stockan, J.A., Taylor, A.F.S. & Woodward, S. 2014. The potential ecological impact of ash dieback in the UK. *Joint Nature Conservation Committee, Report No. 483.* Peterborough 2014.

Morand, M-E., Brachet, S. Rossignol, P. and Dufour, J. 2002. A generalized heterozygote deficiency assessed with microsatellites in French common ash populations. Molecular Ecology 11: 377 – 385.

Sansford, C. E. 2013. Pest Risk Analysis for *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*) for the UK and the Republic of Ireland. Forestry Commission. May 24th 2013. 128pp.

https://secure.fera.gov.uk/phi/riskRegister/plant-health/documents/hymenoscyphusPseudoalbidusPRA.pdf (accessed 20th November 2015).

Thomasset, M. 2011 Introduced hybrid ash: *Fraxinus excelsior* X *F. angustifolia* in Ireland and its potential for interbreeding with native ash. Ph D. Thesis (2011), University of Dublin Trinity College, Ireland.

Thomasset, M., Fernández-Manjarrés, J. F., Douglas, G. C., Bertolino, P. Frascaria-Lacoste, N. and Hodkinson, T. R. 2013. Assignment testing reveals multiple introduced source populations including potential ash hybrids (*Fraxinus excelsior* X *F. angustifolia*) in Ireland. *European Journal of Forest Research* 132: 195-209. http://rd.springer.com/article/10.1007%2Fs10342-012-0667-9.

Thomasset M., Fernández-Manjarrés, J.F., Douglas G.C., Frascaria-Lacoste, N., Raquin, C. and Hodkinson, T.R. 2011a. Molecular and morphological characterization of reciprocal F_1 hybrid ash (*Fraxinus excelsior F. angustifolia*, Oleaceae) and parental species reveals asymmetric character inheritance. *International Journal of Plant Sciences* 17: 423-433. Stable URL: http://www.jstor.org/stable/10.1086/658169.

Thomasset,M., Fernández-Manjarrés, J.F., Douglas, G.C., Frascaria-Lacoste, N. and Hodkinson, T.R. 2011b. Hybridisation, introgression and climate change: a case study for the tree genus *Fraxinus* (Oleaceae). In: *Climate Change, Ecology and Systematics*, ed. Trevor R. Hodkinson, Michael B. Jones, Stephen Waldren and John A. N. Parnell. Published by Cambridge University Press© The Systematics Association 2011 pp. 320-342.

Timmermann, V., Borja, I., Hietala, A. M., Kirisits, T. and Solheim, H. 2011. Ash dieback: pathogen spread and diurnal patterns of ascospore dispersal with special emphasis on Norway. Bulletin OEPP/EPPO 41: 14 - 20

Zhao Yan-Jie , Tsuyoshi Hosoya, Hans-Otto Baral, Kentaro Hosaka & Makoto Kakishima 2012. *Hymenoscyphus pseudoalbidus*, the correct name for *Lambertella albida* reported from Japan. *Mycotaxon* 122: 25-41.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 140 – 149.

From the Alps to the Apennines: possible spread of ash dieback in Mediterranean areas

L. GHELARDINI[1], D. MIGLIORINI[1,2], A. SANTINI[1], A. L. PEPORI[1], G. MARESI[3], N. VAI[4], C. MONTUSCHI[4], E. CARRARI[2], M. FEDUCCI[2], P. CAPRETTI[1,2], N. LUCHI *[1]

[1]Institute for Sustainable Plant Protection, National Research Council (CNR-IPSP), I-50019, Sesto Fiorentino (FI), Italy, [2] Department of Agrifood Production and Environmental Sciences, University of Florence, I-50144, Firenze, Italy, [3] FEM- Centre for Technology Transfer, Via E. Mach 1 I-38010- San Michele all'Adige (TN), Italy; [4] Regional Phytosanitary Services Emilia-Romagna Region, Via di Corticella, 133 I-40128 Bologna Italy.
** corresponding author: nicola.luchi@ipsp.cnr.it*

Abstract

The causal agent of ash dieback, the ascomycetous fungus *Hymenoscyphus fraxineus,* was isolated from *Fraxinus excelsior* in the Apennines Mountains in central Italy. Infected trees showed severe symptoms of ash dieback such as dieback of twigs and branches, and characteristic necroses on shoots, leaves, rachises and petioles. Typical *H. fraxineus* colonies were obtained from symptomatic tissues and identified from morphological and cultural traits as well as by means of molecular analyses. The pathogen was isolated both from potted plants in nurseries and from trees in nearby forests, which confirms that the spread of *H. fraxineus* is largely human-mediated. This is currently the southernmost record of the pathogen in the Italian peninsula and in Europe, indicating that the fungus is able to survive and infect *F. excelsior* in those parts of the geographic range that border areas with Mediterranean climate. *H. fraxineus* has not been isolated so far from *F. ornus* despite the symptoms of decline observed on this species in the study area. However, as suggested by recent studies, *F. ornus* and other species of Oleaceae might be suitable for growth of *H. fraxineus* and might be colonised by the fungus when growing in stands or communities bordering *F. excelsior* stands Such species might contribute, if infected, to increase the reservoir of inoculum of the pathogen in these areas. This newly updated southernmost limit of the distribution of *H. fraxineus* might serve as a possible bridge for disease outbreak in other ash species or for host jump to other species of Oleaceae that are characteristic of the Mediterranean landscape.

Keywords: *Hymenoscyphus fraxineus*, *Fraxinus* spp., spreading front, Oleaceae, forest tree nurseries, optimal climatic range, Mediterranean region.

Introduction

Hymenoscyphus fraxineus (T. Kowalski) Baral, Queloz and Hosoya, recently renamed from the previous *H. pseudoalbidus* Queloz, Grünig, Berndt, Kowalski, Sieber & Holdenr., is a new fungal species to science, with putative origin in eastern Asia (Gross et al. 2014a, Zhao et al. 2012), known as the causal agent of ash dieback, a severe condition that is currently affecting both *Fraxinus excelsior* and *F. angustifolia* in Europe. The pathogen causes damages on different organs and tissues, including necrosis at the base and midrib of leaves, necrosis of rachises, dieback of shoots, twigs or main stem resulting in crown dieback, bark lesions, and sapwood discoloration (Kräutler and Kirisits, 2012). The disease can affect ash trees of any age in forests growing under diverse conditions, in the landscape, in urban plantations and in nurseries (Gross et al. 2014b; Kräutler and Kirisits, 2012; Schumacher et al., 2010).

To date, *H. fraxineus* has only been found to infect ash. Both European ash (*F. excelsior*) and narrow-leaved ash (*F. angustifolia*) are very susceptible (Gross et al. 2014b). The flowering ash (*F. ornus* L.) can be infected when plants are artificially exposed to heavy disease pressure (Kirisits and Schwanda, 2015), but natural infections have never been reported.

Studies indicate that susceptibility varies within European ash populations and quantitative genetic resistance to the disease is found although at low frequency (Enderle et al. 2015, Lobo et al 2014, McKinney et al. 2011). There are no data about variation in resistance in the other European *Fraxinus* species. Studies also suggest that susceptibility may be increased in European ash plants with low fitness (McKinney et al. 2012; Lobo et al 2014). Relations between resistance and host phenology, i.e. timing of bud burst and leaf senescence, have been reported in several studies (Pliura and Baliuckas 2007, McKinney et al. 2011, Kirisits and Freinschlag 2012, Bakys et al. 2013, Pliura et al. 2011, 2014, 2015).

The life cycle of *H. fraxineus* is still partly hypothetical and unsolved (Dvorak et al. 2016), but according to current well-established knowledge the disease is transmitted exclusively by ascospores, which are produced during the growing season in apothecia formed on the leaf litter and dispersed in the air (Gross et al. 2012, Bengtsson et al. 2012, Kowalski et al. 2013). Once landed on susceptible hosts, viable ascospores germinate in favourable conditions and produce new infections penetrating ash leaves via appressoria (Timmermann et al 2011, Clearly et al 2013, Gross et al 2014b).

During the last two decades, *H. fraxineus* has been rapidly spreading across the European continent. Today, the fungus is established in the majority of European countries where ash is present, while has still restricted distribution at the southern and northern range margins (EPPO 2015). In Italy, dieback of *F. excelsior* has been observed since 2009 in the northeastern part of the peninsula, on the southern slope of the Alps. *H. fraxineus* was first reported along the border with Slovenia in July 2009 (Ogris et al. 2010) but later on it spread rapidly in surrounding areas of Friuli-Venezia Giulia and Veneto, reaching Trentino in 2012 (Frigimelica and Maresi 2012) and later also Lombardy in 2014 (Tantardini A., comm. pers.).

The rapid spread of *H. fraxineus* throughout continental Europe is explained by wind dispersal of abundant airborne ascospores in the continuous distribution of the host (FRAXIGEN 2005) upon long-distance movement of infected saplings from tree nurseries (Kirisits et al. 2009; Chandelier et al. 2014; Timmermann et al. 2011). Commercial trade of nursery plants is probably the most common and dangerous dispersal pathway of *H. fraxineus*, which enable easy crossing of geographical barriers and arrival in isolated host populations.

In the Mediterranean Basin, climatic conditions frequently are, both in space and in time, outside the optimal growth range of *H. fraxineus*, mostly because of high temperature and low humidity during summer. On the basis of average requirements of the pathogen in temperature and humidity in the range of the pathogen known at the time, and mean monthly values of climatic variables and soil moisture in summer months, and elevation in the parts of Europe still free from the disease, a recent modelling analysis has classified the whole Iberian Peninsula, Greece and Central to Southern Italy as areas with extremely low risk of ash dieback, although the Authors caution about transferring predictions from the regional to the local scale (Dal Maso and Montecchio 2014). However, suitable conditions for establishment of the pathogen may be locally present especially in northern regions of Mediterranean countries and in the mountains. Moving southwards, sites with such environmental conditions become more scattered and geographically isolated, which renders unlikely the arrival of the pathogen by natural dispersal, but would not prevent disease establishment in case of human-mediated introduction. In Italy, as well as in Spain and Greece, where the pathogen has not been reported sites with suitable conditions for establishment of the pathogen and outbreak of the disease may be present, especially in the mountains, depending on elevation and local topography.

Until 2012, *H. fraxineus* was neither reported on *F. excelsior* nor *F. angustifolia* in the Apennines (Luchi et al., 2012). In these mountains, differently from Central and Northern Europe, European ash has a scattered natural distribution (Bernetti 1995). Narrow-leaved ash (*F. angustifolia*) is found on relatively drier sites at altitudes up to 2000 m, as well as on riverine and wetland sites (FRAXIGEN 2005). Both these ash species are common in mixed forest with conifers or other broadleaves. Moreover, in medium or low elevation areas of the Apennines, *F. ornus* and *Olea europea* grow in neighbouring habitats with *F. excelsior*. In such instances, airborne propagules of the pathogen could intercept new host plants that might be susceptible to ash dieback.

During the past few years, the Regional Phytosanitary Services of Tuscany and Emilia Romagna have taken measures to prevent possible outbreaks of ash dieback in the Northern Apennines, which are the closest mountains to the Alps, where the pathogen is established since several years. Monitoring surveys were planned to assess the health status of *Fraxinus* species (*F.excelsior*, *F. angustifolia*, and *F. ornus*) and monitor the possible introduction of *H. fraxineus* in these Regions. This paper summarizes monitoring actions carried out in 2014 and 2015 in Central Italy (Regions of Emilia-Romagna and Tuscany). Despite all efforts, the pathogen has been introduced and recently detected in both regions, both in natural stands and nurseries, a finding that have much extended southwards the distribution of *H. fraxineus* in the Mediterranean Basin. Isolation and identification of the fungus from common ash (*F. excelsior*) in the Apennines is also described.

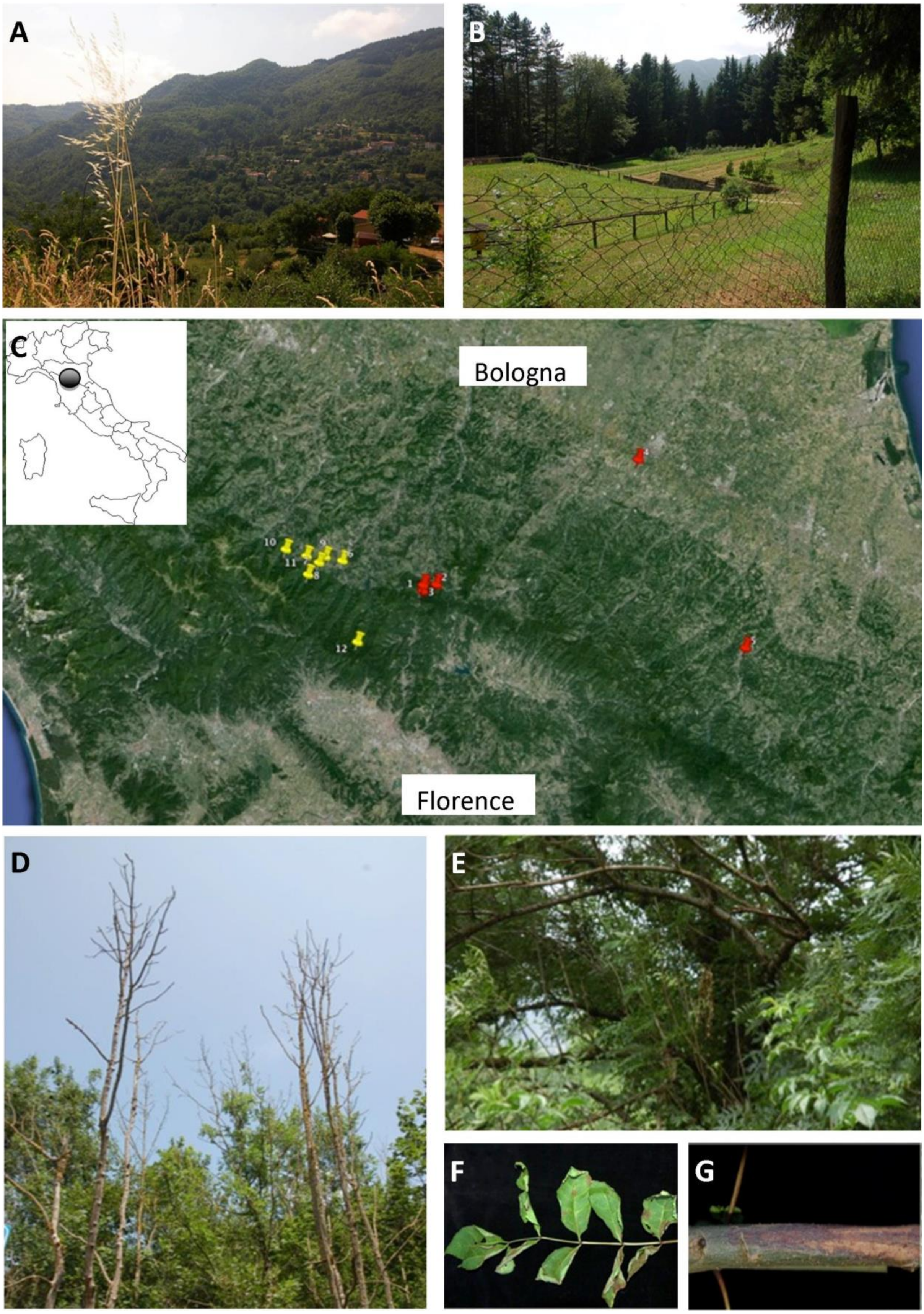


Figure 1 (see next page for descriptions)

Figure 1A) Typical landscape in the Northern Apennines in Montepiano (Province of Prato, Central Italy) where *Fraxinus excelsior* grows in mixed forests with conifers and other Oleaceae. **1B)** Regional Forest nursery "Le Cottede" (Castiglion dei Pepoli, Province of Bologna, Central Italy) where *F. excelsior* potted seedlings were found infected by *Hymenoscyphus fraxineus*. **1C)** Sampling sites of *Fraxinus excelsior* and *F. ornus* in Central Italy along the border between the Regions Tuscany and Emilia-Romagna. Red: site where ash dieback has been observed and *Hymenoscyphus fraxineus* has been detected. Yellow: site where ash dieback has not been observed. Sampling sites are listed in Table 1. Image provided by Google Earth. **1D-1G)** Symptoms of ash dieback caused by *Hymenoscyphus fraxineus* on *Fraxinus excelsior* in Montepiano (Province of Prato, Central Italy): 1D**)** Dead and dying ash trees; 1E**)** Mature tree on the roadside with dying shoots in the lower crown; 1F**)** typical necroses along leaf midribs and on leaf blades; 1G**)** Stem necrosis on a tree;

Material and Methods

Sampling sites

The survey was carried out in 2014 and 2015 in nurseries and forests at 12 different sites in the Northern Apennines or in the proximity of the mountains, in the Regions of Tuscany and Emilia-Romagna (Central Italy) (Table 1, Fig.1). Mean annual precipitation and temperature were obtained from the closest meteorological stations of the Regional weather forecasting network (http://www.lamma.rete.toscana.it and http://www.arpa.emr.it). The sites had mean annual temperature in the range 9.2-14.3 °C, and mean annual precipitation in the range 922-1940 mm (Table 1). Samples were collected from plants of *F. excelsior* and *F. ornus* showing symptoms of decline and growing in natural forests or along roadsides (Table 1). Additional samples were collected from *F. excelsior* potted plants in three Forest Tree Nurseries in Emilia-Romagna (Table 1). The samples were transferred to the laboratory to ascertain the presence of *H. fraxineus*.

Isolation and identification of *H. fraxineus*

Twigs and branches showing symptoms of ash dieback and leaf rachises from the litter were collected at each sampling site (Table 1). The samples were surface sterilized (Kräutler and Kirisits 2012). Fragments cut from the margins of putatively infected tissue were placed on 90mm Petri dishes containing 2% malt extract agar (MEA, Oxoid). After incubation at 22 °C for 15 days fungal colonies resembling to *H. fraxineus* were sub-cultured onto MEA and the strains were conserved in the collection of the IPSP-CNR. The pathogen was identified by means of morphological comparison to a reference isolate (KAB9, kindly provided by T. Kirisits).

Fungal genomic DNA was extracted from 100 mg fresh mycelium growing on 300 PT cellophane discs placed on ash leaves medium in 90mm Petri dishes (Carrari et al., 2015). After 10 days the mycelium was harvested and DNA extraction was carried out using the EZNA Plant DNA Kit (Omega Bio-tek). Amplification of the Internal Transcribed Spacer region (ITS) of the rDNA, including the 5.8S gene, PCR amplified with the primers ITS1 and ITS4 (White *et al*., 1990). The amplification was done using the following protocol: 95°C for 5 min, initial denaturation; 35 cycles of 94°C for 1.30 min., 56°C for 1 min., 72°C for 2 min; and 72°C for 10 min final extension. PCR products were purified using the mi-PCR Purification kit (Metabion International, Germany) and sequenced at Macrogen (Seul, South Korea). The sequences were blasted against the sequences deposited in GenBank using the BLAST function in NCBI (http://blast.ncbi.nlm.nih.gov).

Presence of *H. fraxineus* in symptomatic tissues was confirmed by real-time qPCR. Small fragments of putatively infected wood (c. 100 mg fresh weight) were transferred to 2-ml microfuge tubes with two tungsten beads (3 mm) and 0.6-ml buffer lysis P1 (EZNA Plant DNA Kit), and ground with a Mixer Mill 300 (Qiagen) (2 min; 20 Hz). DNA was extracted from all samples using the EZNA Plant DNA Kit (Omega Bio-tek) according to manufacturer's instructions. The real-time qPCR assay specific for *H. fraxineus* developed by Ioos et al. (2009) was applied for detecting the pathogen.

Table 1: Characteristics of sampling sites in Northern Apennines (Italy).

	Sampling site	**Latitude** **Longitude**	**Host species**	**Characteristics of the infected plants**	**Temperature* (°C)**	**Precipitations** (mm)**
1	Montepiano (Province of Prato)	44° 5’ 34” N 11° 09’ 30” E	*Fraxinus excelsior*	Ash trees growing on roadsides mixed with *Robinia Pseudoacacia* or on riversides mixed with *Alnus spp.*	10.4	864
2	Monte Tavianella (Province of Bologna)	44° 06’ 31.4” N 11° 11’ 1.3” E	*F. excelsior*	Ash trees growing in small pure stands or in the margin of Douglas fir stands along the roadside	11.8	1281
3	Cottede (Province of Bologna)	44° 06’ 36.9” N 11° 09’ 56.1” E	*F. excelsior*	Regional Forest Nursery, potted plants	11.8	1281
4	Zerina di Imola (Province of Bologna)	44° 19’ 19.1” N 11° 40’ 9.8” E	*F. excelsior*	Regional Forest Nursery, potted plants	14.3	731
5	Castellaro di Galeata (Province of Forlì Cesena)	43° 57’ 31.4” N 11° 54’ 54.0” E	*F. excelsior*	Regional Forest Nursery, potted plants	12.4	922
6	Porretta Terme (Province of Bologna)	44° 09' 12.7" N 10° 58' 16.9" E	*F. ornus*	Small stands growing on roadsides	10.9	1462
7	Madonna del Faggio (Province of Bologna)	44° 08' 7.7" N 10° 53' 54.3" E	*F. excelsior*	Small stands growing on roadsides	9.9	1590
8	Canevaio (Province of Bologna)	44° 08' 55.8" N 10° 55' 28.4" E	*F. excelsior* & *F. ornus*	Small stands growing on roadsides	9.9	1590
9	Castellaro (Province of Bologna)	44° 09' 32.1" N 10° 56' 23.1" E	*F. ornus*	Ash trees mixed with *Pinus nigra* and *Robinia pseudoacacia*	10.9	1462
10	Madonna dell’Acero (Province of Bologna)	44° 09’ 1.2” N 10° 49’ 26.9” E	*F. excelsior*	Small ash stands mixed with *Acer spp.*	9.9	1590
11	Lizzano in Belvedere (Province of Bologna)	44° 09’ 50.4” N 10° 53’ 56.8” E	*F. ornus*	Ash trees mixed with *Pinus nigra*	9.9	1590
12	Acquerino (Province of Bologna)	43° 59' 11.9" N 10° 59' 59.4" E	*F. excelsior*	Small ash stands mixed with *Acer spp.*	9.2	1940

*Mean annual temperature for the period 1991-2008;
**Mean annual total precipitation (1991-2008).

Results and Discussion

Ash dieback symptoms were observed in adult trees and saplings of *F. excelsior* at Montepiano (site n. 1) and at Monte Tavianella (site n. 2) (Table 1; Figure 2). The trees showed extensive shoot dieback (Figure 3). Shoots had necrotic bark lesions and cankers (Figure 3). Longitudinal sections of twigs and shoots revealed necroses in cambial tissues and in the xylem. Symptomatic potted plants were found in all surveyed forest tree nurseries (sites n. 3,4,5 ; Figure 2). All observed symptoms were consistent to those reported in the literature for infection by *H. fraxineus* (Kräutler and Kirisits, 2012; Ogris et al., 2010).

Both morphological evaluation of the colonies and molecular analyses confirmed the presence of *H. fraxineus* in symptomatic tissues of *F. excelsior*. Conidial morphology and morphological features of cultures were like those of the reference strain and corresponded to the description of *H. fraxineus* in the literature (Kräutler and Kirisits, 2012). Isolates resembling to *H. fraxineus* formed cottony colonies, white to orange-brown (Figure 4). These cultures were incubated at 5°C in order to induce production of sporulating mycelium. After 5 weeks incubation dark-grey mycelia containing conidiophores with conidia (Figure 4) were observed in the margins of colonies. Identification of *H. fraxineus* was confirmed by sequencing representative isolates at the ITS rDNA region. BLAST searches in the NCBI database revealed a 100% homology with *H. fraxineus* sequences deposited in GenBank. The ITS sequence of the reference specimens isolated from European ash from site n.1 and site n.2 in the Apennines (strains CNRHfrax300, CNRHfrax301, CNRHfrax24.4, CNRHfrax51.1) were deposited in GenBank (accession number KT696592, KT696593, KT696594 and KT696595). All isolated strains are conserved in the culture collection of the Institute for Sustainable Plant Protection (IPSP-CNR, Sesto Fiorentino, Florence, Italy) (Luchi et al., 2016).
In the present survey ash dieback due to infection by *H. fraxineus* was detected in some stands very close to forest tree nurseries (sites n.1-2) and also in some potted seedlings in nurseries (site 3,4,5). The plants were destroyed afterwards. However, in the other stands included in the survey (n.7, 8, 10, 12 - Table 1) *F. excelsior* trees resulted healthy and did not show any symptom of decline. Although severe symptoms of ash decline, including dieback in the apical parts of the shoots (Figure 5) were observed in *F. ornus*, *H. fraxineus* was never isolated from this species.

Movement of plants between different geographic areas by commercial trade or other human activities, represent the primary pathway of introduction of exotic and possibly invasive pathogens (Santini et al., 2013). When an alien pathogen arrives to a region where native host species have little resistance, sudden and destructive epidemics can be triggered with severe impact on the environment and economy (Brasier 2008). This has been the case with spread of *H. fraxineus* in Italy where forest nurseries have produced plant material for landscape and soil protection from the end of 19th century until recently. During the last decades, the activity of forest nurseries belonging to Regions has been important to preserve the germplasm of Mediterranean trees and shrubs and to protect biodiversity. These nurseries grow also plants of non-local origin, among them *F. excelsior* is largely used. Importation of infected plant stocks by forest nurseries might therefore be the cause of the recent spread of the pathogen to neighbouring natural stands in the Northern Apennines. In addition the massive airborne dispersal ability of the pathogen and the climatic conditions favourable to *H. fraxineus* infections, have allowed the establishment of the disease this new area.

The effect of environmental factors is crucial in the plant disease triangle, as the combination of susceptible host and virulent pathogen does not drive disease unless environmental conditions are favourable. Based on *in vitro* culture of pure mycelial colonies, *H. fraxineus* can be classified as a mesophile and cold tolerant microorganism, which has on average maximum growth at 20-22°C, extremely limited or absent growth at 28-30°C, reduced growth at 5°C, but is still able to cause necrosis and produce phialides at low temperature (Hauptman et al. 2013, Kowalski and Bartnik, 2010). Therefore, the capability of the pathogen to infect host trees and survive in European regions with warmer climate has been questioned. It should be noticed, however, that there is quite large variation in optimal growth temperature even among isolates from relatively uniform areas, for instance up to 30% of tested isolates had either higher (25°C) or lower (15°C) optimum temperature in Poland (Kowalski and Bartnik, 2010). Hauptman and colleagues (2013) have studied heat tolerance of the fungus in culture. Mycelial cultures tolerate up to 24 h with no damage or only minor (10%) damage at 34 and 36°C respectively. Exposure to 38°C for 10 h was tolerated with limited injure (20% of the colonies did not re-grow after treatment). Four hours at 40°C and at 42°C were required to kill half and 2/3 of the fungus, respectively, but only after 8 and 16h of continuous exposure to these temperatures were all colonies dead. The fungus was killed by 1h exposition to 44°C. There were differences in heat tolerance between isolates.

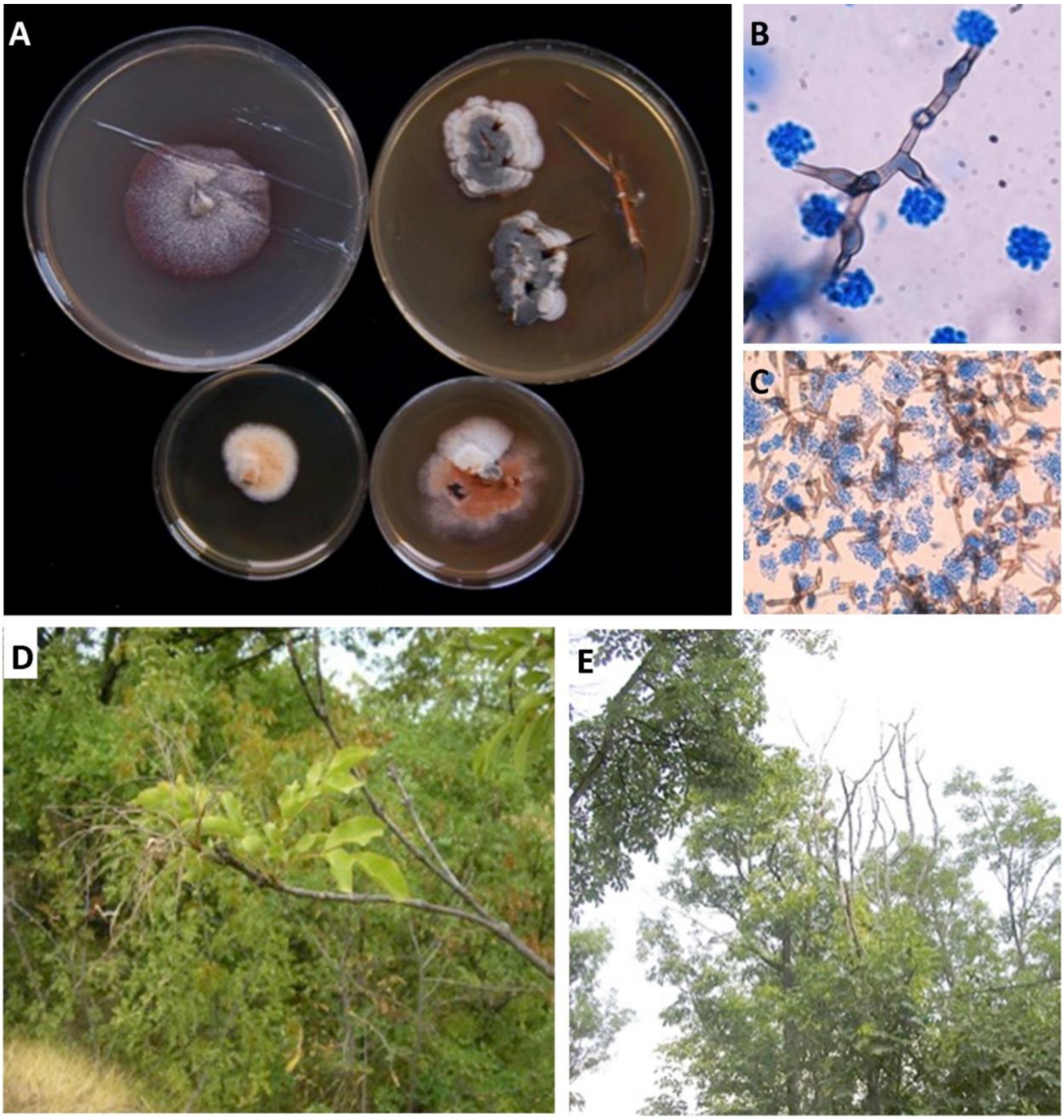


Figure 2A) *Hymenoscyphus fraxineus* colonies and fungal hyphae: upper left - mycelium growing on MEA supplemented with ash leaves after 15gg at 20°C; upper right - stromatic mycelium on MEA after 4weeks at 5°C; lower left - mycelium after 13 days at 20°C on MEA; lower right - mycelium after 27 days at 20°C on MEA. **2B – 2C)** Phialophores and conidia formed on stromatic mycelium. **2D – 2E)** Symptoms of ash dieback on *Fraxinus ornus* (Lizzano in Belvedere, Province of Bologna; Central Italy). *Hymenoscyphus fraxineus* has never been isolated from this species.

Temperatures in cambium and wood tissues inside a forest canopy depend on air temperature, wind and exposure to direct light, greatly vary with plant height, and can be higher than air temperature at certain times and local positions (Emerson 1898, Derby and Gates 1966). However, on the basis of data on heat tolerance reported above, *H. fraxineus* seems to be able to survive with limited damages even under the hot summer conditions of Mediterranean areas inside twigs and trunks of host trees in the forests.

Dispersal of *H. fraxineus* is mostly due to ascospores that are considered drought sensitive and are abundantly produced during several months in late spring and summer (Gross et al. 2014b). Apothecia production on ash leaf rachises in the litter is favoured by moist soil conditions (Gross et al. 2014b). The typical Mediterranean climate provides two wet periods, in spring and fall, very favourable for production of ascospores. If the fungus is able to survive and reproduce under conditions of summer drought and high temperatures, it might potentially spread to suitable areas in the Mediterranean basin.

Rapid establishment and spreading of *H. fraxineus* in the study area in the Northern Apennines was probably enabled by local climatic conditions quite similar to those found in the Alps and central Europe. Vegetation and climatic studies position the transition area between temperate cold and Mediterranean regions approximately between the northern Apennine ridge and the Mount Conero near Ancona (Pignatti, 1979, Brunetti et al., 2004, Piovesan et al., 2005). Finding of the fungus in this ecotone might be the prelude to spreading of the disease towards new host plants from the Mediterranean region. In some areas of the northern Apennines broadleaved species form communities where *F. excelsior* nearby *F. ornus* (Bernetti 1995). At the southernmost limit, the distribution range of *F. excelsior* is in contact with the northern range of *F. angustifolia* and *F. ornus*. The last species is largely represented in the central-southern part of the peninsula, including Sicily and Sardinia (Bernetti 1995). In these areas suitable environmental conditions might allow the pathogen to infect new and closely related host species (host jump) in the same genus or family, such as *F. angustifolia* and *F. ornus*. Moreover, some studies have shown that other Oleaceae, mainly present in the Mediterranean area but often growing in stands or communities bordering *F. excelsior* or *F. ornus* stands, might be suitable hosts for *H. fraxineus*, and might be threatened by introduction of this new pathogen in these areas (Carrari et al., 2015; Madigan et al., 2015).

To preserve and protect the biodiversity of ash trees and other Oleaceae species in Mediterranean areas, more detailed information about this new invasive pathogen and the adoption of a more stringent European legislation on plant trade are needed, in order to predispose friendly mitigation strategies against this and other alien threats which are mostly of anthropogenic origin. In this context accurate monitoring actions combined with early molecular detection techniques of the pathogen, would be crucial to prevent disease spread to new areas and host species, including those characterizing the Mediterranean landscape, at the southern boundary of the current distribution range.

Acknowledgements

The research was supported by a grant of Regione Toscana “Monitoraggio sulla eventuale presenza di Chalara fraxinea, valutazione del rischio di diffusione del patogeno fungino in boschi e vivai con presenza di frassino della Regione Toscana” (cod ZEF0979C8B), and by COST Action FP1103 “Fraxinus dieback in Europe: elaborating guidelines and strategies for sustainable management (FRAXBACK)”.

References

Bakys, R., Vasaitis, R., Skovsgaard J.P. 2013. Patterns and severity of crown dieback in young even-aged stands of European ash (*Fraxinus excelsior* L.) in relation to stand density, bud flushing phenotype, and season. Plant Protection Science 49: 120-126.

Bengtsson, S.B.K., Vasaitis, R., Kirisits, T., Solheim, H., Stenlid, J. 2012. Population structure of *Hymenoscyphus pseudoalbidus* and its genetic relationship to *Hymenoscyphus albidus*. Fungal Ecol 5: 147-153.

Bernetti, G. 1995. Selvicoltura speciale. UTET, Torino. Pp.415.

Brasier, C.M. 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathology 57, 792-808.

Brunetti, M., Buffoni, L., Mangianti, F., Maugeri, M., Nanni, T. 2004.Temperature, precipitation and extreme events during the last century in Italy. Global Planet. Change 40, 141–149.

Carrari, E., Capretti, P., Santini, A., Luchi, N. 2015. *Hymenoscyphus fraxineus* mycelial growth on media containing leaf extracts of different Oleaceae. Forest Pathology 45: 540-543.

Chandelier, A., Helson, M., Dvorak, M., Gischer, F. 2014. Detection and quantification of airborne inoculum of *Hymenoscyphus pseudoalbidus* using real-time PCR assays. Plant pathology, 63(6), 1296-1305.

Cleary, M.R., Daniel, G., Stenlid J. 2013. Light and scanning electron microscopy studies of the early infection stages of *Hymenoscyphus pseudoalbidus* on *Fraxinus excelsior*. Plant Pathol 62: 1294-1301.

Dal Maso, E. and Montecchio, L. 2014. Risk of Natural Spread of *Hymenoscyphus fraxineus* with Environmental Niche Modelling and Ensemble Forecasting Technique. Forest Research: Open Access, 2014.

Derby, R.V. and Gates, D.M. 1966. The temperatures of tree trunks-calculated and observed. American Journal of Botany 53(6):580-587.

Dvorak, M., Rotkova, G., Botella, L. 2016. Detection of airborne inoculum of *Hymenoscyphus fraxineus* and *H. albidus* during seasonal fluctuations associated with absence of apothecia. Forests 7, 1; doi:10.3390/f7010001.

Emerson, R.A. 1898. On the internal temperature of tree trunks. Transactions and reports of the Nebraska State Historical Society, Series 2 Vol. 2, Lincoln, Neb.: The Society, 1894-1902, pp. 245-250.

Enderle, R., Nakou, A., Thomas, K., Metzler, B. 2015. Susceptibility of autochthonous German Fraxinus excelsior clones to *Hymenoscyphus pseudoalbidus* is genetically determined. Annals of Forest Science 72(2): 183-193.

EPPO. 2015. Global Database, *Hymenoscyphus fraxineus* (CHAAFR), Distribution: https://gd.eppo.int/taxon/CHAAFR/distribution (last updated: 2015-11-18).

FRAXIGEN. 2005. Ash Species in Europe: Biological Characteristics and Practical Guidelines for Sustainable Use. University of Oxford, Oxford. 128 p.

Frigimelica, G. and Maresi, G. 2012. Il deperimento del frassino. Terra trentina, 58 (4): 54.

Gross, A., Hosoya, T., Queloz, V. 2014a. Population structure of the invasive forest pathogen *Hymenoscyphus pseudoalbidus*. Molecular Ecololgy 23, 2943–2960.

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V., Sieber, T. N. 2014b. *Hymenoscyphus pseudoalbidus*, the causal agent of ash dieback. Molecular Plant Pathology 15: 109-117.

Gross, A., Zaffarano, P.L., Duo, S. and Grünig, C.R. 2012. Reproductive mode and life cycle of the ash dieback pathogen *Hymenoscyphus pseudoalbidus*. Fungal Genet Biol 49: 977-986.

Hauptman, T., Piškur, B., de Groot, M., Ogris, N., Ferlan, M., Jurc, D. 2013. Temperature effect on *Chalara fraxinea*: heat treatment of saplings as a possible disease control method. Forest Pathology 43: 360-370.

Ioos R, Kowalski T, Husson C, Holdenrieder O. 2009. Rapid in planta detection of *Chalara fraxinea* by a real-time PCR assay using a dual-labelled probe. Eur. J. Plant Pathol. 125: 329-335.

Kirisits, T., Matlakova, M., Mottinger-Kroupa, S., Cech, T. L., Halmschlager, E. 2009. The current situation of ash dieback caused by *Chalara fraxinea* in Austria. In Proceedings of the conference of IUFRO working party (Vol. 7, No. 02, pp. 97-119).

Kirisits, T. and Freinschlag, C. 2012. Ash dieback caused by *Hymenoscyphus pseudoalbidus* in a seed plantation of *Fraxinus excelsior* in Austria. Journal of Agricultural Extension and Rural Development 4(9): 184-191.

Kirisits, T. and Schwanda, K. 2015. First definite report of natural infection of *Fraxinus ornus* by *Hymenoscyphus fraxineus*. Forest Pathology, 45(5), 430-432.

Kowalski, T. and Bartnik, C. 2010. Morphologial variation in colonies of *Chalara fraxinea* isolated from ash (*Fraxinus excelsior* L.) stems with symptoms of dieback and effects of temperature on colony growth and structure. Acta Agrobot. 63, 99–106.

Kowalski, T., Białobrzeski, M., Ostafińska, A. 2013. The occurrence of *Hymenoscyphus pseudoalbidus* apothecia in the leaf litter of Fraxinus excelsior stands with ash dieback symptoms in southern Poland. Acta Mycologica, 48(2), 135-146.

Kräutler, K. and Kirisits, T. 2012. The ash dieback pathogen *Hymenoscyphus pseudoalbidus* is associated with leaf symptoms on ash species (Fraxinus spp.). J. Agric. Ext. Rural Dev. 4, 261–265.

Lobo, A., Hansen, J.K., McKinney, L.V., Rostgaard Nielsena, L., Kjær, E.D. 2014. Genetic variation in dieback resistance: growth and survival of *Fraxinus excelsior* under the influence of *Hymenoscyphus pseudoalbidus*. Scandinavian Journal of Forest Research 29(6): 519-526.

Luchi, N., Montecchio, L., Santini, A. 2012. Situation with Ash in Italy: stand characteristics, health condition, ongoing work and research needs. In: Interim Report from *Chalara fraxinea*, FRAXBACK Meeting in Vilnius, 13–14 November 2012 (Mainprize, N., Hendry, S. and Weir, J., eds), pp. 25–26. Bristol: Forestry Commission.

Luchi, N., Ghelardini, L., Santini, A., Migliorini, D., Capretti, P. 2016. First record of ash dieback caused by *Hymenoschyphus fraxineus* on *Fraxinus excelsior* in the Apennines (Tuscany, Italy). Plant Disease, 100: 2, 535.

Madigan, A., Belka, M., Taylor, A.F.S., Kirisits, T., Cleary, M., Nguyen, D., Elfstrand, M. and Woodward, S. 2015. Can *Hymenoscyphus fraxineus* infect hardy members of the Oleaceae other than ash species? Forest Pathology 45(5): 426-429.

McKinney, L.V., Nielsen, L.R., Hansen, J.K., Kjær, E.D. 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (Oleraceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. Heredity 106(5): 788-797.

McKinney, L. V., Thomsen, I. M., Kjær, E. D., Nielsen, L. R. 2012. Genetic resistance to *Hymenoscyphus pseudoalbidus* limits fungal growth and symptom occurrence in *Fraxinus excelsior*. Forest Pathology, 42(1), 69-74.

Ogris, N., Hauptman, T., Jurc, D., Floreanci,g V., Marsich, F., Montecchio, L. 2010. First report of *Chalara fraxinea* on common ash in Italy. Plant Disease: 94(1), p. 133.

Pignatti, S., 1979. I piani di vegetazione in Italia. Giornale Botanico Italiano 113, 411–428.

Piovesan, G., Biondi, F., Bernabei, M., Di Filippo, A., Schirone, B. 2005. Spatial and altitudinal bioclimatic zones of the Italian peninsula identified from a beech (*Fagus sylvatica* L.) tree-ring network. Actoa Oecologica 27:197-210.

Pliura, A., Baliuckas, V. 2007. Genetic variation in adaptive traits of progenies of Lithuanian and western European populations of *Fraxinus excelsior* L. Baltic Forestry 13 (1): 28-38.

Pliura, A., Lygis, V., Suchockas, V., Bartkevičius, 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.

Pliura, A., Marčiulyniene, D., Bakys, R., Suchockas, V. 2014. Dynamics of genetic resistance to *Hymenoscyphus pseudoalbidus* in juvenile *Fraxinus excelsior* clones. Baltic Forestry 20 (1): 10-27.

Pliura, A., Lygis, V., Marčiulyniene, D., Suchockas, V., Bakys, R. 2015. Genetic variation of Fraxinus excelsior half-sib families in response to ash dieback disease following simulated spring frost and summer drought treatments. iForest 9: 12-22.

Santini, A., Ghelardini, L., Pace, et al. 2013. Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytologist,197(1), 238-250.

Schumacher, J., Kehr, R., Leonhard, S. 2010. Mycological and histological investigations of *Fraxinus excelsior* nursery saplings naturally infected by *Chalara fraxinea*. Forest Pathology, 40: 419-429.

Timmermann, V., Børja, I., Hietala, A. M., Kirisits, T., Solheim, H. 2011. Ash dieback: pathogen spread and diurnal patterns of ascospore dispersal, with special emphasis on Norway. EPPO Bulletin, 41(1), 14-20.

White, T.J., Bruns, T., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A Guide to Methods and Applications. (Ed. by M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White), pp. 315–322. Academic Press, San Diego (CA), USA.

Zhao, Y.-J., Hosoya, T., Baral, H.-O., Hosaka, K., Kakishima, M. 2012. *Hymenoscyphus pseudoalbidus,* the correct name for *Lambertella albida* reported from Japan. Mycotaxon, 122, 25–41.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 150 – 165.

Ash dieback in Lithuania: disease history, research on impact and genetic variation in disease resistance, tree breeding and options for forest management

A. PLIŪRA[1,2]*, R. BAKYS[3], V. SUCHOCKAS[1,2], D. MARČIULYNIENĖ[1], A. GUSTIENĖ[3], V. VERBYLA[4], V. LYGIS[5]

[1]Department of Forest Genetics and Breeding, Department of Silviculture, Institute of Forestry, Lithuanian Research Centre for Agriculture and Forestry, [2]Faculty of Forestry and Ecology, Aleksandras Stulginskis University, [3]Department of Forest Sanitary Protection, Lithuanian State Forest Service, [4]Department of Forest Genetic Resources, Lithuanian State Forest Service, [5]Laboratory of Phytopathogenic Microorganisms, Institute of Botany of Nature Research Centre
**Corresponding author: alfas.pliura@mi.lt*

Abstract

In the presented review we aimed to summarize the existing knowledge on ash dieback and possible options of its control (forest management and tree breeding) accumulated in Lithuania over the last 20 years of the ongoing epidemic. Due to this devastating disease Lithuania has lost more than a half of its forest stands where *Fraxinus excelsior* was a dominant tree species, and health condition of the remaining stands is still continuously deteriorating. According to data collected from permanent forest monitoring plots, about 9% of *F. excelsior* trees die out every year. Several research projects have been dedicated to investigate reasons and consequences of the current ash dieback phenomenon; also, several tree breeding projects targeted to detect and breed disease-resistant *F. excelsior* genotypes, families and populations have been launched. The review presents most important results of the implemented research projects and most important findings from Lithuanian *F. excelsior* progeny and clonal trials which revealed substantial genetic variation and heritability of susceptibility/resistance traits and indicated a good potential of selection and breeding of dieback-resistant ash propagation material. In addition, we briefly present studies on communities of xylotrophic fungi residing in declining ash trees, and genetic diversity, population structure and virulence of *H. fraxineus* isolates of Lithuanian origin. Finally, we present a summary of researcher's recommendations prepared for Lithuanian forest owners and managers for management of dieback-affected ash stands and for selection of *F. excelsior* genotypes least susceptible to *H. fraxineus* and their further use for establishment of resistant seed orchards and further tree breeding for resistance. Future research needs include studies of ash regeneration potential in forest stands, and revealing genetic diversity in and structure of the naturally regenerating *F. excelsior* populations. Moreover, studies of natural forest succession in dieback-affected but not further managed *F. excelsior* stands are highly desired. As options of effective management of the devastated stands and means for disease control are still quite limited, the research will focus on one of the most promising options to save ash - its selection and breeding for resistance against *H. fraxineus*.

Keywords: common ash (*Fraxinus excelsior*), stand decline, genetic variation, *Hymenoscyphus fraxineus* (*Chalara fraxinea*), forest regeneration, health (sanitary) condition, tree breeding

Introduction

Distribution and importance of common ash in Lithuania

In Lithuania, common ash (*Fraxinus excelsior* L.) is the only naturally growing ash species; here it forms unique broadleaved ecosystems and occurs mostly in mixed stands, while pure *F. excelsior* stands are (or were before the ash dieback epidemic) also not exceptional. *F. excelsior* thrives in rich wetland forests where it grows in mixture with *Alnus glutinosa* (L.) Gaertn., *Alnus incana* (L.) Moench, *Betula pendula* Roth., *Populus tremula* L. and *T. cordata* Mill. (Narbutas, 1975). The main factors delimiting natural distribution of *F. excelsior* are the lack of its tolerance to a prolonged draught, winter cold and late spring frosts; on the other hand, this tree is adaptive to a wide range of environmental conditions, exhibiting intermediate properties between a pioneer species and a permanent forest component (Pliūra 1999, Pliūra & Heuertz 2003, Dobrowolska et al. 2011). Because of prolific natural regeneration, stands dominated by *F. excelsior* in Lithuania were (before the ash dieback epidemic) usually reforested naturally, although artificial reforestation of clear-felled areas with nursery-grown ash seedlings was also occasionally used (Forest State Service, personal communication).

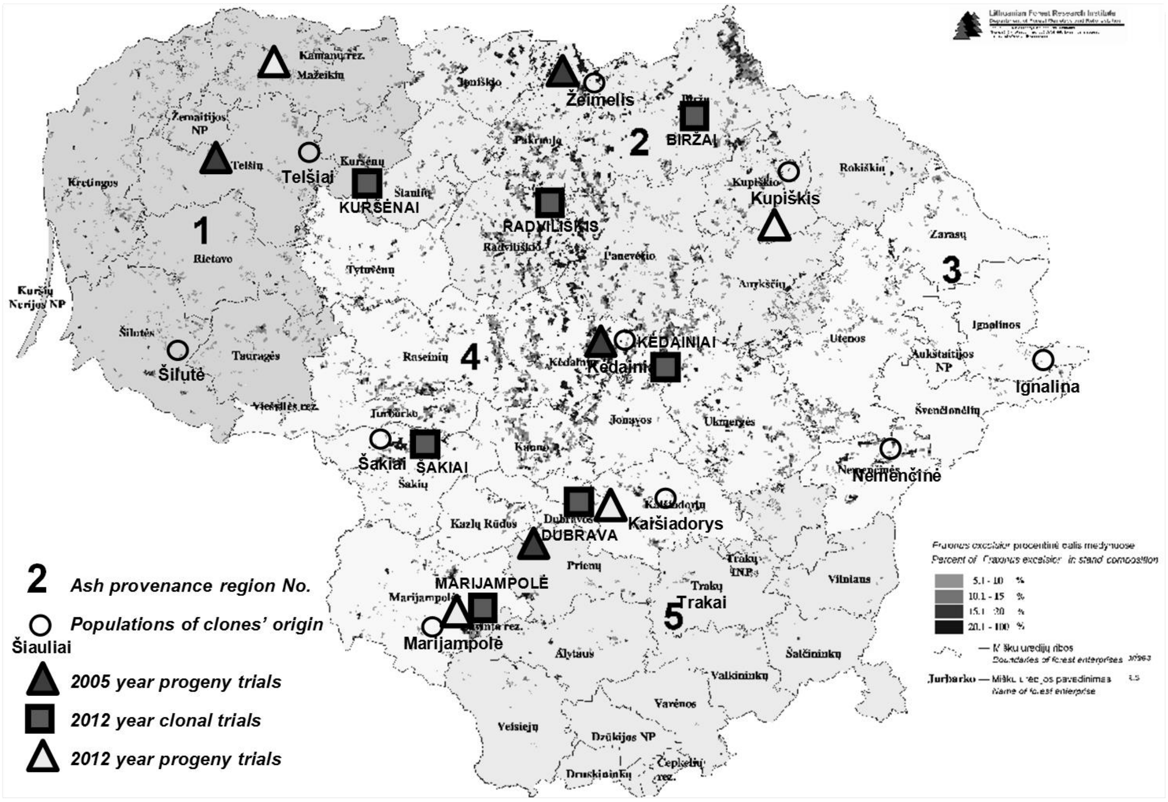


Figure 1 Distribution of *Fraxinus excelsior*-dominated stands (small green patches of various intensity), natural *F. excelsior* populations sampled for establishment of various progeny trials (open circles), established progeny trials (triangles), and clonal trials-seed orchards (squares) of *F. excelsior* in Lithuania. Provenance regions of *F. excelsior* are indicated with different background colours.

Ash-dominated stands are distributed mainly in the Lithuanian Middle Plain and northern part of the country, but smaller patches occur all over Lithuanian territory (Figure 1) with a larger proportion of ash on rich and moist soils. Although share of *F. excelsior* in the total growing stock is small, this tree species is regarded economically important in Lithuania: its wood is mainly used for production of furniture, wood floors (parquet), interior facing and firewood, yet today the demand of ash timber further and further exceeds its supply. Common ash is considered to be one of the key landscape tree species, although its role in this respect is probably less important than in many Western European countries. During previous century the area of *F. excelsior*-dominated stands in Lithuania has been gradually increasing due to active ash-oriented forestry on rich and moist soils. For example, there was a notable increase of ash stand area in the second half of the previous century: this area has grown from about 16.1 thous. ha in 1962 to 52.7 thous. ha in 2000 (Verbyla et al. 2003). This trend of increase however got interrupted by ash dieback epidemic which emerged in Lithuania at the end of the XXth century.

Current statistics of ash stands in Lithuania

Since the turn of the XXth century the ash dieback epidemic has caused dramatic losses in *F. excelsior* stock across the country. According to Lithuanian national forest inventory data (Anonymous 2015), in 2015, the total growing stock volume in ash-dominated stands was 4.0 mln. m^3, mean growing stock volume– 170 m^3/ha, mean gross annual increment – 5.7 m^3/ha; mean wood accumulation was negative – -9.9 m^3/ha, mean stocking level – 41% and mean age – 69 years. In comparison, in 2000, the respective values were as follows: total growing stock volume – 7.6 mln. m^3, mean growing stock volume – 150 m^3/ha, mean gross annual increment – 5.7 m^3/ha; mean wood accumulation – 3.5 m^3/ha, mean stocking level – 68% and mean age – 49 years (Anonymous 2001). It is obvious that the quantitative characteristics of Lithuanian ash stands have been shifted down dramatically over the last 15 years. Although over this period the mean growing stock volume per ha in ash-dominated stands slightly increased and mean gross annual increment remained the same, the total growing stock volume of ash has dramatically decreased by nearly a 3.6 million cubic meters, which was mainly due to intensified sanitary fellings and decreased wood accumulation rates. The current mean stocking level in ash stands (43%) is considered critically low, and because of an unacceptable density the stands are often subjected to sanitary clear-fellings (according to Lithuanian Rules on Forest Fellings (2015), stands damaged by pests and diseases with a stocking level of 40% and below are subjected to sanitary clear-fellings). It must be pointed out also that current age structure of the remaining ash stands is changing towards a larger share of older trees as no new ash plantations are being established, and natural regeneration in the devastated stands is largely unsatisfactory (Lygis et al. 2014, Lithuanian State Forest Service, personal communication). For example, in 2000, distribution of ash stands by age classes was as follows: young, 42%; middle-aged, 50%; premature, 6%, and mature, 2%, while in 2015 the respective percentages were 15, 77, 4 and 4%. Due to continuing sanitary fellings, area of *F. excelsior* stands has decreased from 52.7 thous. ha in 2001 (the maximum ash stand area registered since the beginning of the previous century) to 24.1 thous. ha in 2015, i.e., from 2.7% to 1.2% of all Lithuanian forest area (Anonymous 2001, 2015; Figures 2 & 3).

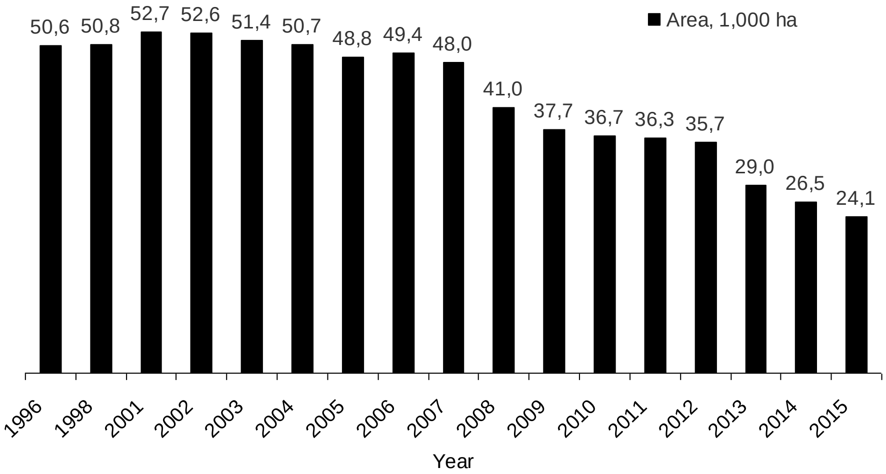


Figure 2 Dynamics of *Fraxinus excelsior* stand area in Lithuania over 1996–2015 (Lithuanian State Forest Service, personal communication)

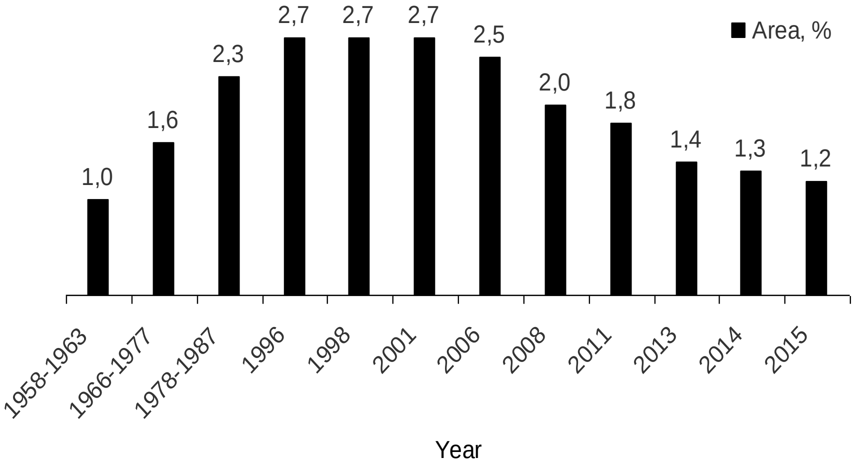


Figure 3 Dynamics of share of *Fraxinus excelsior* stand area in a total Lithuanian forest stand area over 1958–2015 (Lithuanian State Forest Service, personal communication)

History and consequences of the ash dieback epidemic in Lithuania

Since 1992, a severe dieback of common ash has spread from Eastern Poland to most European countries, including Lithuania (Gross et al. 2014, and references therein). In Lithuania, the dieback of *F. excelsior* has emerged around 1996 in forests of North-Central Lithuania and soon expanded across the whole country (Juodvalkis and Vasiliauskas 2002, Gustienė 2010). Currently the epidemic is in its chronic phase, large areas of

Lithuanian ash stands are already felled by selective or clear sanitary fellings (Figure 4), and virtually all remaining ash stands are damaged to a greater or lesser extent, and their health condition continues to deteriorate (Pliūra et al. 2011, Lygis et al. 2014, Lithuanian State Forest Service, personal communication).

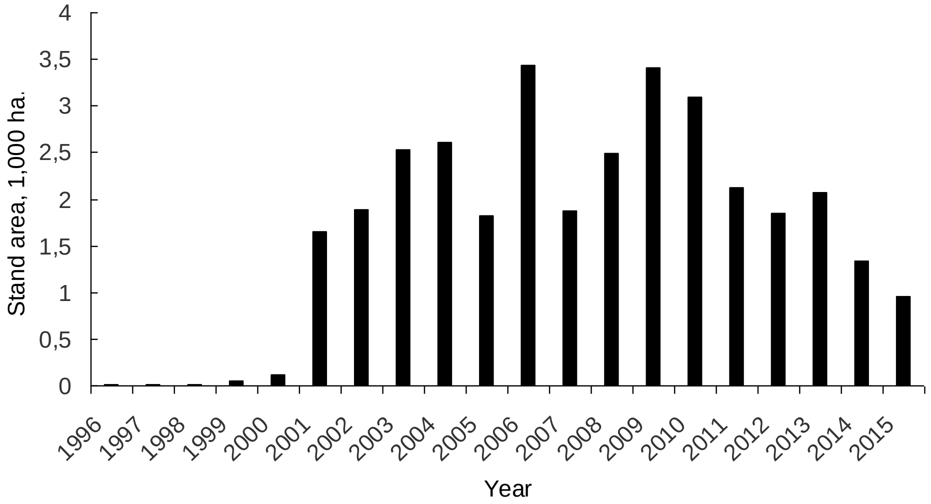


Figure 4 Area of *Fraxinus excelsior* stands felled by selective and clear sanitary fellings in Lithuanian state-owned forests over 1996–2015 (Lithuanian State Forest Service, personal communication)

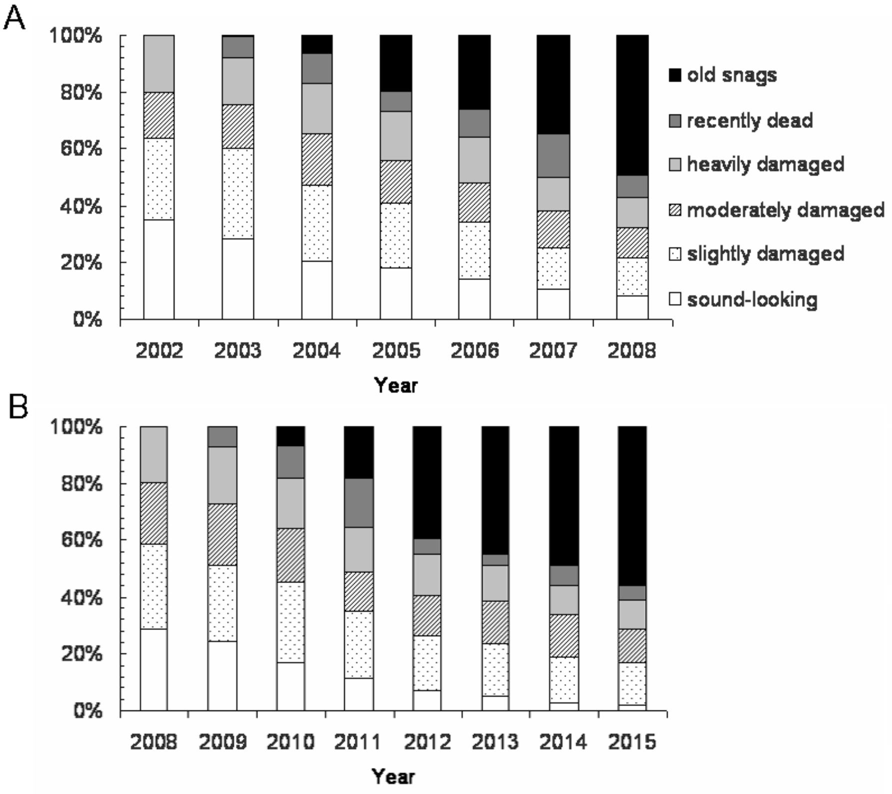


Figure 5 Distribution of *Fraxinus excelsior* trees by health condition categories in two sets of permanent monitoring plots established in Joniškis, Nemenčinė, Raseiniai, Šakiai and Ukmergė State Forest Enterprises. A, mean tree condition in 25 plots established in 2002 (here, the monitoring has been terminated in 2008), and B, mean tree condition in 20 plots newly established in 2008 (Lithuanian State Forest Service, personal communication)

To monitor dynamics of sanitary state of *F. excelsior*, in summer of 2002, a total of 25 permanent monitoring plots have been established by Lithuanian Forest Sanitary Protection Service in forests of Joniškis, Nemenčinė, Raseiniai, Šakiai and Ukmergė State Forest Enterprises which represent different regions of the country. The stands were selected randomly among those with a minimum fraction of ash of 70% and mean age of ash trees ranging between 40 and 90 years. Each plot included from 28 to 59 *F. excelsior* trees (1,218 trees in total or 48.7 trees per plot on average) of various sanitary condition – from sound-looking (defoliation 0–25%) to heavily damaged (defoliation 76–99%). The monitoring in these plots was terminated in 2008 due to high ash mortality rates: 692 or 56.8% of the monitored trees died in six years (9.5% per year on average). Tree condition was gradually deteriorating each year: for example, the number of sound-looking trees has decreased from 422 (34.6%) in 2002 to 101 (8.3%) in 2008 meaning that on average about 4.4% of the sound-looking trees were damaged by the dieback each year (Figure 5A).

In summer of 2008, 20 new permanent monitoring plots have been established in the same State Forest Enterprises, mostly in the same forest stands with similar characteristics, although this time the plots were set in stand areas least damaged by the dieback. Each plot included from 30 to 55 *F. excelsior* trees (857 trees in total or 42.9 trees per plot on average) of various sanitary condition – from sound-looking to heavily damaged. Over seven-year period of observation (2008–2015), 524 (61.1%) of the monitored *F. excelsior* trees died indicating that on average annual mortality rate remained very similar to that observed in previously monitored plots (8.7%). Again, the same trend of deterioration of tree condition was observed (Figure 5B): the number of sound-looking trees decreased from 244 (28.5%) in 2008 to 16 (1.9%) in 2015 (on average, about 3.8% of sound-looking trees got damaged each year). These results clearly indicate gradual and continuous deterioration of ash sanitary condition in Lithuania, however the ongoing intensive natural selection creates a good basis for selection of least susceptible individuals for resistance breeding.

Ash trees are subjected to dieback in forest stands, seed plantations, clonal archives, progeny trials and forest nurseries (Pliūra et al. 2011, 2014, 2016, Lygis et al. 2014, Marčiulynienė et al. 2015). To date, no clear correlation was found between the extent of mortality and tree age, species composition, geographic position of stands or edaphic factors (soil moisture and fertility) (Juodvalkis and Vasiliauskas 2002, Lygis et al. 2014, Lithuanian State Forest Service, personal communication). Before the main causal agent of ash dieback, an invasive ascomycete *Hymenoscyphus fraxineus* (T. Kowalski) Baral et al. (anamorph *Chalara fraxinea* T. Kowalski, syn. *Hymenoscyphus pseudoalbidus* Queloz et al.) has been identified (Kowalski 2006), there were lots of speculations on possible causes of *F. excelsior* decline in Lithuania. Among the discussed likely causes were not only pathogenic fungi (e.g., *Armillaria* spp., *Nectria coccinea* Desm. (=*Neonectria coccinea* (Pers.) Rossman & Samuels), *N. galligena* (Bres.) Rossman & Samuels, *Gleosporium* sp., *Cytophoma pulchella* (Sacc.) Gutner) (Juodvalkis and Vasiliauskas 2002), but also mechanical ash stem damages – bark peeling wounds made by wild game (Padaiga 2002), increased ozone pollution and general tree weakening caused by various environmental stress factors primarily driven by a global climate change (Ozolinčius 2002). Of the abiotic stress factors that could be involved, an increased frequency of dry years and a lowered level of ground water were among the most frequently mentioned (Juodvalkis and Vasiliauskas 2002, Ozolinčius 2002, Skuodienė et al. 2003, Karpavičius and Vitas 2006). Yet, most of the researchers agreed that this new phenomenon was a consequence of simultaneous action of a complex of various adverse factors.

Today nobody doubts that the primary cause of the present dieback is continual infections of ash crowns by *H. fraxineus*, while root rot caused by a secondary pathogen *Armillaria* spp., observed virtually in every ash stand (Lithuanian State Forest Service, personal communication), plays none the less important role. Commonly occurring root rot caused by *Armillaria* spp. is regarded one of the main reasons of premature death and/or uprooting of *F. excelsior* trees in Lithuania (Lygis et al. 2005, Bakys et al. 2011). Bakys et al. (2011) demonstrated that the extent of root and butt rot in declining *F. excelsior* positively correlates with a severity of crown dieback caused by *H. fraxineus*.

Due to the ongoing dieback and continuous deterioration of ash health condition there is also a big loss in areas used for conservation of Lithuanian *F. excelsior* genetic resources: the one and the only seed stand and 7 out of 11 former ash genetic reserves are already gone (written off due to high mortality rates). Progeny studies have shown that proportion of ash trees with a high level of natural resistance is currently very low, probably less than 5% (Pliūra et al. 2011). Today, density of the remaining viable and flowering *F. excelsior* trees is extremely low (3–5 trees per hectare) in most of the remaining (yet, severely damaged) ash stands. It is very likely that an effective population size (N_e) has already decreased substantially thus compromising the remaining level of genetic diversity and possibilities for genetically sufficient regeneration of *F. excelsior* populations (Pliūra et al. 2011). Natural ash regeneration is very poor both in terms of quantity and quality regardless of time since stand felling, fraction of *F. excelsior* in a former (pre-dieback) stand, site conditions (forest site type) or geographic location (Lygis et al. 2014). As establishment of new ash plantations in Lithuania has been suspended already for more than a decade, poor current recruitment rate and health condition of *F. excelsior* self-regeneration is not likely to ensure domination of this tree species in future stands. In most cases ash is largely outnumbered and suppressed by early-successional tree species such as grey alder *Alnus incana* (L.) Moench), birch (*Betula pendula* Roth. and *B. pubescens* Ehrh.) and common aspen (*Populus tremula* L.) leading to a significant shift in species composition as compared to the situation in pre-dieback stands (Lygis et al. 2014).

Research on ash dieback and tree breeding projects

Lithuanian researchers are involved into activities of three ongoing research projects, targeted or associated with ash dieback: 1) collaborative Lithuanian-Swiss Cooperation Programme project “Incidence of mycoviruses in epidemic and post-epidemic populations of the ash dieback pathogen *Chalara fraxinea* and evaluation of their potential for biological control of the disease (CONTROLDIEBACK)” (2012–2016), 2) Lithuanian National Programme project "Response and plasticity of different tree species & juvenile-stage forest communities under impact of climate change and other environmental stressors" (2015–2018; a part of the project assigned to studies of genetic variation in regeneration of *F. excelsior* in severely damaged stands *in situ* and studies of its plasticity and competitiveness with other tree species under stress impacts in a controlled environment), and 3) Irish-Lithuanian-French-Norwegian-UK’s collaborative project "Research required to investigate genetic resistance to ash dieback disease *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*), and the development of disease resistant ash planting stock" (2016–2020; WP3: 3.1.3 of the Long Term Forest Research CoFoRD Programme). Lithuanian researchers and representatives from the forestry sector are involved also into activities of the COST programme Action No. FP1103 “*Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management (FRAXBACK)” (2012–2016).

Completed research projects: 1) an international SNS – Nordic Forest Research Co-operation Committee project “Decline of *Fraxinus excelsior* in northern Europe” (2010–2012; task of the Lithuanian team - investigation of potential of *F. excelsior* self-regeneration following sanitary clear-fellings in dieback-affected ash stands), 2) Lithuanian National Programme project „Vulnerability of native tree species and populations, changes of natural distribution range and forecasts for climate changes“ (2010–2011; a part of this project was dedicated to analysis of overall situation in Lithuanian ash stands and genetic variation of *F. excelsior* in disease resistance); 3) national research project „Progeny-based evaluation of *F. excelsior* populations and plus trees for disease resistance, selection of the most resistant genotypes, and preparation of recommendations for establishment of seed orchards” (2010), 4) national research project “Eco-genetic control of resistance to the pathogen *Chalara fraxinea* in common ash” (2012–2014), and 5) Lithuanian National Programme project „Vulnerability and productivity of local species under biological invasions and climate change” (2012–2014; a part of the project was dedicated to analysis of *F. excelsior* stand decline in Lithuania). Also, a four-year PhD study project on ash dieback (studies on epidemiology, genetic structure and virulence of *H. fraxineus* populations of different origin aiming to evaluate perspectives of further growing and breeding of *F. excelsior*) has been initiated and successfully accomplished (PhD student D. Marčiulynienė; 2011–2015).

Self-regeneration potential in devastated *F. excelsior* stands

In north-temperate forest stands, *F. excelsior* is (or, better say, used to be) highly competitive in mixed species regeneration both in openings and under the forest canopy (Narbutas 1975, Tapper 1993, Dobrowolska et al. 2011). Before the outbreak of ash dieback epidemic, *F. excelsior* was regarded as an extremely efficient clear-cut colonizer on moist, well-drained, nutrient-rich calcareous sites: in Lithuania, the density of ash regeneration used to reach up to 100,000 individuals ha^{-1} or even more (Narbutas 1975). Under normal conditions, *F. excelsior* produces large numbers of well dispersed seeds and is adaptive to a wide range of environmental conditions (FRAXIGEN 2005, Dobrowolska et al. 2011). Moreover, vegetative regeneration from fresh ash stumps was usually prolific that allowed using coppicing for formation of new ash-dominated stands as an alternative to planting (Narbutas 1975, Wagner 1990, FRAXIGEN 2005). Bakys et al. (2011) investigated stump sprouting potential in three ash clear-cut sites in South-Western Lithuania and found that majority of *F. excelsior* stump sprouts exhibited typical symptoms of *H. fraxineus*-caused dieback or were dead irrespective of presence/absence of stump decay primarily caused by *Armillaria* spp. The authors came to a conclusion that in dieback-affected areas vigorous natural ash regeneration by stump sprouting should not be expected because of frequent infections by *H. fraxineus*.

In 2011, quality and quantity of forest self-regeneration was assessed in areas that were initially dominated by *F. excelsior*, but were subsequently devastated by the ash dieback disease, and as a result subjected to sanitary clear-felling (Lygis et al. 2014). The inventory of forest regeneration (both originating from seeds and stump sprouting) was performed on 20 sites located in Biržai (Northern Lithuania) and Raseiniai (South-Western Lithuania) State Forest Enterprises, clear-felled 1–10 years previously. The results of this study showed that regeneration of *F. excelsior* in examined clear-cuts was poor both in quantitative and qualitative terms: ash proportion decreased from 40–100% in pre-dieback stands to 0–21% in current regeneration where ash has been outnumbered by birch, grey alder and aspen. Moreover, only about 1/3 of the regenerating ash trees were visually healthy. It has been concluded that this will inevitably lead to a sharp decrease in the proportion of ash comprising the total species composition of regenerating stands. In the investigated sites, health condition of *F. excelsior* seedlings was significantly better than that of stump sprouts, indicating that potential for selecting breeding material both from the regenerating clear-cuts and adjacent (seed) stands is evident, and such selection should be considered as an important means for ash conservation in the future.

Research in population, progeny and clonal trials of *F. excelsior*

First studies on disease development and genetic variation in resistance and other adaptive traits of *F. excelsior* have been performed in three progeny trials established in 2005 in three different ash provenance regions of Lithuania under cooperation with European Commission's Fifth Framework Programme (FP5) project "RAP - Realizing Ash Potential. Improving *Fraxinus* (Ash) productivity for European needs by testing, propagation and promotion of improved genetic resources" (2001–2005) (Pliūra and Baliuckas 2007, Pliūra et al. 2011, Figure 1). In these trials, a total of 140 open-pollinated families from ten Lithuanian populations and 180 open-pollinated families from fourteen foreign European (Belgian, Czech, Danish, French, German, Irish and Polish) populations are being tested. The primary results of progeny testing in these trials are summarized in Pliūra et al. (2011) and in Douglas et al. (2013). Briefly, significant differences in resistance to the ash dieback disease among tested *F. excelsior* populations and among families have been found. None of the tested families showed complete resistance to ash dieback, and only a fraction of the families exhibited reduced susceptibility. Similar results have been obtained in West-European clonal trials where only a small fraction of *F. excelsior* clones exhibited good disease resistance, while the majority of clones showed increasingly high disease incidence rates (McKinney et al. 2011, Kirisits and Freinschlag 2012, Stener 2013). Obtained high additive coefficients of genetic variation (29.9-38.7%) and heritability estimates (h^2 = 0.40–0.49) for health condition indicated that resistance is genetically predetermined and inheritable, and allows forward selection and breeding of the resistant genotypes. It was found that health condition (in terms of degree of damage and survival rate) of foreign *F. excelsior* populations was in general worse (with a few exceptions) than that of the Lithuanian populations. This possibly indicated a more advanced stage of natural selection in dieback-affected Lithuanian ash populations compared to the mostly disease-free foreign populations at the time of trial establishment. The obtained results clearly indicated that introduction of foreign populations to restore Lithuanian ash stands may have a doubtful potential and should be considered with care.

Based on estimated BLUP breeding values, complex breeding indexes and so-called 'Pyramid selection principle' (McDonald and Linde 2002a,b; Baniulis et al. 2008), 50 superior individuals were selected within best families from the best populations for establishment of resistant *F. excelsior* breeding populations in different provenance regions of Lithuania, as well as for establishment of clonal trials and seed orchards of second generation. Picking out 50 least susceptible trees from the total number of the planted 27,000 individuals means the extreme selection intensity (one out of the outplanted 540 trees, i = 0.185%) that shall result in a high genetic gain in resistance.

In 2012, a clonal archive and 6 clonal trials-seed orchards have been established aiming to test the performance (growth parameters and resistance to *H. fraxineus*) of the 50 progeny-tested most resistant *F. excelsior* clones, 28 of which represented Lithuanian and 22 – foreign populations (Pliūra et al. 2011), and additional 180 clones selected for resistance in natural *F. excelsior* populations across Lithuania (Figure 1). The performance of ash clones in these trials has been assessed in 2012–2013 (Pliūra et al. 2014). Briefly, the overall incidence of ash dieback disease has increased considerably already in one year following trial establishment (from 10.7% disease incidence observed in 2012 to 72.2% in 2013). In 2012, dieback symptoms were observed on 45.9% of the tested clones, while in 2013 all tested clones were to a greater or lesser extent damaged by the disease. Coefficients of clonal genetic variation (vc_c) and broad-sense heritability (H^2) in *F. excelsior* varied year-by-year but remained rather high during fifth and sixth growing seasons of the tested clones. This indicated substantial and permanent genetic control of the disease resistance. The results of this study also showed that a large proportion of *F.*

excelsior clones initially considered resistant, in a longer run (or in different environmental conditions) may show a certain degree of susceptibility raising serious concern of foresters and tree breeders as regards existence of a *sensu stricto* genetic resistance in common ash. Nevertheless, disease incidence in the tested 50 clones remained significantly lower than in the base population of half-sib families in progeny trials thus indicating a certain success of the first cycle of selection for resistance against *H. fraxineus*. Health condition score of parent individuals significantly negatively correlated with disease incidence in clones ($r = -0.36$, $P = 0.01$) and positively correlated with health condition of clones ($r = 0.36, P = 0.01$), indicating that clones produced from healthier individuals were healthier than ones from susceptible individuals. Significant genotype by environment interaction (G × E) was found for most of tree damage traits (mortality rates, disease incidence and intensity of damage). Similar or even higher G × E variance component values were obtained for most of the tree damage traits compared to values obtained for tree height and bud flushing phenology. This indicates a presence of genetic variation in plasticity and reaction norms of ash clones across sites providing that resistance to disease of the most susceptible clones unequally depends on environmental conditions.

In 2012, four new progeny trials have been established for testing 36 families from two Lithuanian populations (Biržai and Žeimelis (Pakruojis), Figure 1). In 2014, 20 of these families were tested in climatic chambers under the impact of simulated spring frost and summer drought treatments (Pliura et al. 2016). The results of this study showed significant among-family variation in disease incidence and health condition in each treatment. This pointed to an additive mode of gene action, or, in other words,- to a quantitative resistance to the disease. A hypothesis has been raised that such resistance might be durable in a long term as it combines different plant defence mechanisms, thereby diminishing the probability of breaking the resistance due to mutation or adaptation of the pathogen. Population effect for any of the assessed resistance traits was non-significant which was in a good agreement with results reported by Olrik et al. (2007), Stener (2007), McKinney et al. (2011) and Pliūra et al. (2014). The absence of significant population effect could be due to a high among-family variation within the tested populations which number was in general quite low. The genotype by environment interaction (family × treatment) was found to be significant for disease incidence, length of necrotic shoots and tree survival implying that susceptibility of ash families to the dieback disease unequally depends on environmental conditions, and indicating a presence of genetic variation in plasticity and reaction norms of the tested families across environments (treatments) (Pliūra et al 2016). Different levels of damage among the tested families in two stress-induced events (spring frost and summer drought treatments) and control indicated variable adaptive potential of different families, and warranted testing of material across range of environments in tree breeding for resistance.

In general, health condition scores and seedling survival ratio showed rather strong positive correlations with bud flushing phenology scores. It has been concluded that early-flushing clones are less susceptible to the ash dieback disease. This is in agreement with the results of other studies which showed that bud flushing and (or) leaf senescence phenology exhibit a moderate to strong correlation with dieback susceptibility (Pliūra and Baliuckas 2007, McKinney et al. 2011, Kirisits and Freinschlag 2012, Bakys et al. 2013, Pliūra et al. 2011, 2014). Interestingly, in our study bud flushing phenology score moderately negatively correlated with mean seedling height: the early flushing families were of smaller height growth but showed lower susceptibility to the disease. This study showed substantially increased coefficients of additive genetic variation and heritability for health condition following both frost and drought treatments (Pliūra et al 2016). Such simulated stress conditions may noticeably contribute to expression of tree traits which are used to rank tested ash individuals, families or populations for their susceptibility to the dieback. Subsequently, this should enable a better evaluation of the performance of different families, effective family selection, and achievement of a marked genetic gain.

Further testing of 20 above-mentioned *F. excelsior* families for their susceptibility to ash dieback pathogen has been carried out trough artificial inoculation with a virulent strain of *H. fraxineus* (Marčiulynienė et al. 2015). The results of this study showed that families from Pakruojis (Žeimelis) population were of significantly better health condition compared to families from the geographically close Biržai population. Statistically significant differences in all parameters reflecting susceptibility to the dieback disease were also detected among the tested families. Length of necroses developed at inoculation point clearly reflected susceptibility of the investigated ash populations and families to the dieback. This finding corresponded well with the results obtained by Danish researchers McKinney et al. (2012) and Lobo et al. (2015), and in general may allow improvement of selection efficacy of resistant ash genotypes, families and populations. Detection of statistically significant differences in susceptibility among ash genotypes of different origin shows good potential in selection and breeding of resistant ash propagation material.

Studies on communities of xylotrophic fungi in declining ash trees

Communities of fungi inhabiting *F. excelsior* stems and roots and their possible impact on tree health condition were investigated by Lygis et al. (2005) and Bakys et al. (2011). Lygis et al. (2005) reported principally different fungal communities in stem bases (root collar) of 210 ash trees of different health condition growing within the same forest stand (Northern Lithuania). In total, 15 sound-looking, 132 declining and 63 dead trees from three discrete plots yielded 352 isolates, representing 75 fungal taxa. Although similar numbers of species were found in wood of sound-looking, declining and dead *F. excelsior* trees, the shift in the fungal community structure was considerable indicating that fungal species in wood of living trees are likely to change along with changes in tree condition. *Armillaria cepistipes* Velen. was found to be the dominant fungus in all tree health categories; its population structure has been determined in the three study sites by vegetative incompatibility tests which showed the prolonged presence of some genotypes of the fungus (at least for 20–30 years) in the investigated stands without causing any serious damage. A hypothesis therefore has been raised that recently-started ash decline might be associated with observed crown damages caused by some yet unknown microorganism (which later appeared to be *H. fraxineus*). Actually, during this study *H. fraxineus* has been isolated from root collars of several sound-looking and declining *F. excelsior* trees, yet at that time the fungus was labelled as *Hymenoscyphus* sp. 970 and regarded only as an endophyte of ash wood. The fungus was correctly identified in 2008 when its DNA sequence was assigned to *H. fraxineus* (GenBank accession no. AY787704, as *Chalara fraxinea*).

Bakys et al. (2011) aimed to identify fungi colonizing roots of dieback-affected *F. excelsior* in South-Western Lithuania, to check their pathogenicity towards ash and to estimate incidence and health condition of vegetative ash regeneration in relation to presence of rot in stumps and root systems. The extent of rot was measured in 33 trees with different dieback intensity, 150 fungal isolations were attempted from roots of 50 trees (this yielded 31 different fungal taxa). A total of 26 fungal taxa were tested for pathogenicity on 286 seedlings of *F. excelsior*, and only few taxa (five basidiomycete species) caused cambium discolorations on some of the inoculated seedlings showing only moderate (if any) pathogenicity. Ash stump sprouting potential was evaluated for 328 stumps on three clear-cut sites. Root rot was mainly caused by *A. cepistipes* which also showed the highest pathogenicity to ash among the tested fungi in the inoculation tests. Four years after tree felling, root rot had a negative impact on sprouting frequency, yet rot did not enter sprouts from stumps, and when experimentally inoculated, fungi associated with root rot in mature ash had no visible impact on tree vigour, showing that after formation of sprouts, rot does not affect the subsequent phytosanitary condition.

In 2012–2013, a large-scale fungal isolation from symptomatic *F. excelsior* stem, branch and shoot pieces was carried out in a frame of the collaborative Lithuanian-Swiss Cooperation Programme project ‘CONTROLDIEBACK’. Sixty trees have been sampled in each of the five ash stands representing different geographic regions of Lithuania (Vilnius, Ukmergė, Kėdainiai, Biržai and Kretinga State Forest Enterprises) (Burokiene et al. 2015). As a result, 809 lesion samples were collected from 300 trees. In addition, two fallen ash leaf petioles with pseudosclerotial plates were collected from forest floor under each sampled tree which in total resulted in 600 petiole samples. Isolation from the collected material yielded 549 *H. fraxineus* isolates, of which 356 were recovered from necrotic lesions and 193 – from leaf petioles.

A collection of isolates of *H. fraxineus* and other *F. excelsior*-associated xylotrophic fungi from all over the country is continuously accumulated and stored in the culture collection of the Laboratory of Phytopathogenic Microorganisms at the Institute of Botany of Nature Research Centre, Vilnius, Lithuania (LPM).

Studies into genetic diversity, population structure and virulence of ash dieback pathogen

Genetic diversity and structure of *H. fraxineus* populations originating from Lithuania and Switzerland were investigated by Burokiene et al. (2015). Briefly, eleven species-specific microsatellite markers (SSR‘s) were employed to investigate and compare pathogen’s populations of different geographic and ecological origin. The investigated populations of the fungus were characterized by high genotypic and low genetic (allelic) diversity and showed no genetic structure. Genetic differences among/between the investigated *H. fraxineus* isolates, subpopulations (representing different stands) and populations (representing two distant countries) were negligible. No genetic differentiation was detected also between *H. fraxineus* isolates representing different ecological phases of its life cycle, i.e. between isolates recovered from necrotic lesions (pathogenic phase) and fallen leaf petioles (saprophytic phase).

To assess and to compare virulence of 200 Lithuanian (100) and Swiss (100) isolates of *H. fraxineus*, artificial stem wound inoculations were conducted on 3-year-old *F. excelsior* seedlings (Lygis et al., submitted). The results of this experiment showed that vast majority of the isolates (98%), regardless their origin, were able to induce necrotic lesions on seedling stems after 10 months post inoculation. Although a high variation in virulence was observed among isolates, no significant differences were detected between the older-established (Lithuanian) and the epidemic-front (Swiss) populations. A hypothesis has been raised that Lithuanian population may still be too young to show significant changes in the pathogen's virulence compared to the Swiss population, or that the availability of the host tree (*F. excelsior*) may not (yet) represent a critical factor for the survival of *H. fraxineus*. The vast majority of *H. fraxineus* isolates showed a strong necrotrophic potential as a bark pathogen on *F. excelsior* regardless of whether they were recovered from leaf petioles or necrotic lesions. This result is consistent with the microsatellite analysis of the same populations of the fungus showing no genetic differentiation and a similar level of genetic diversity (Burokiene et al. 2015).

Forest management to mitigate the impact of the ash dieback

According to general forestry recommendations for management of dieback-affected ash stands prepared for Lithuanian forest owners and managers (Juodvalkis and Vasiliauskas 2002, Juodvalkis 2003, Riepšas 2009) it is advised to cut diseased ash stands by sanitary fellings, although the way of felling (clear-cut or selective) must be selected depending on mean age, proportion and condition of *F. excelsior* trees in a stand, species composition, age structure, stocking level, and presence of understory (focusing on ash regeneration). According to Lithuanian Rules on Forest Fellings (2015), a stand may be subjected to a sanitary clear-felling if its stocking level gets as low as 40% after felling dead and severely damaged trees. Subsequently, large areas have already been clear-felled in stands with a substantial proportion of ash. In sites subjected to the clear-fellings, the most vigorous seed trees (15–25 trees/ha) of *F. excelsior* and of some other target tree species are to be retained (in fact, most of these,- mostly ash and elm trees, die out within a few years because of fungal diseases). Here follows the summary of more detailed management recommendations prepared for ash stands (Juodvalkis 2003, Riepšas 2009):

In undamaged and slightly damaged pure ash stands or ash stands with a minor fraction of other tree species it is recommended to avoid pre-commercial thinnings. If stand stocking level is high, the thinnings may be performed in stands with a lower proportion of ash, yet ash trees should not be regarded as thinning object. Advance regeneration of ash, oak and spruce should be retained and tended with a special attention; in slightly and moderately damaged ash stands of middle or premature age it is recommended to plant oak and spruce seedlings under the forest canopy.

Management of heavily damaged/dying/dead ash stands depends on mean age of *F. excelsior*, its fraction in a stand (tree species composition) and distribution pattern:

- Heavily damaged juvenile, middle-aged and premature mixed stands with a low proportion of *F. excelsior* should be managed by application of group thinnings and/or selective sanitary fellings. If in such stands an upper layer (overstory) is formed of mature aspen, birch, spruce or black alder trees, it is advised to cut the upper layer in one or two cases aiming to increase light availability to advance regeneration and to form mixed uneven-aged stands.

- In dense juvenile stands with ash fraction not exceeding 60%, thinnings are carried out aiming to form a mixed stand consisting of oaks, spruces, birches, black alders and ash of acceptable condition. If a stand is a mixture of ash and low-value tree species, it is recommended to carry out so-called reconstruction fellings by removing the low-value species and planting more valuable species instead. Dead and heavily damaged *F. excelsior* trees may either be removed by selective sanitary fellings or left in the stand as so-called "biodiversity trees", while undamaged and slightly damaged ash trees should always be retained. If ash grows in groups, and following dieback larger open areas are formed, such areas may either be replanted with target tree species (if area of these openings exceeds 50 m^2), or left for a natural regeneration if they cover less than 50 m^2;

- If fraction of heavily damaged *F. excelsior* in a juvenile stand is 70% or more, such stand may be subjected to a sanitary clear-felling. The clear-cut area may either be replanted or left for a natural regeneration (where regeneration of valuable species is likely, usually on wet sites). During the clear-felling target tree species (including ash of an acceptable condition) should always be retained;

- In middle-aged or premature stands with ash fraction not exceeding 50%, selective sanitary fellings should be applied if stand stocking level after the fellings will not fall below 50%; if it falls below 50%, the stand shall be cut by sanitary clear-fellings. If ash is dying in groups and such areas exceed 100 m^2, these are cleaned and replanted by target tree species. If the proportion of heavily damaged *F. excelsior* in a stand is high (exceeds 50%) and other tree species are mature, such stands should be clear-felled seeking to retain viable understory and/or lower canopy of the target tree species. With or without additional planting this allows formation of a mixed stand. In stands which stocking level gets low after removal of damaged/dead ash, it is recommended to establish spruce and/or oak under-canopy plantations;

- Mature ash stands should be subjected to ordinary clear-fellings, although the most vigorous seed trees of *F. excelsior* should be retained. The clear-cut sites should either be replanted with target tree species, or left for a natural regeneration (especially where formation of highly productive birch stands is expected);

Under the presence of abundant and viable understory and/or lower canopy of target tree species, heavily damaged mature ash stands may also be cut by selective sanitary fellings, yet the post-felling proportion of target tree species (other than ash) should be substantial.

In practice, though, these recommendations are not always followed: according to Lithuanian State Forest Service (personal communication), forest owners often make spontaneous decisions on what felling method is to be applied in damaged ash stands (which is usually based on willingness to achieve maximum economic yield) and sometimes pay little attention on retaining viable ash trees. On the other hand, in a large proportion of cases the retained trees lose their viability and eventually die.

Artificial re-establishment of ash is currently not recommended in Lithuania due to a high risk of infections by *H. fraxineus* on newly established plantations. Based on largely reduced interest in buying ash seedlings and high seedling mortality rates observed already in forest nurseries, their production has ceased. Currently only forest tree breeding sector at the Lithuanian State Forest Service together with researchers from the Institute of Forestry of Lithuanian Research Centre for Agriculture and Forestry are carrying out continuous selection of least damaged *F. excelsior* populations and individuals, take scion shoots from the most valuable trees for grafting and further establishment of seed plantations, various trials and clonal archives.

Regulations of reforestation and forest planting have been adjusted in 2008 in favour of planting tree species other than *F. excelsior* on former ash sites; alternatively, these sites may be left for a natural regeneration aiming to form a new stand from retained advance growth and vigorous seedlings of target tree species including ash. However, according to results of clear-cut survey performed in dieback-devastated ash stands, self-regeneration of *F. excelsior* on such sites is very poor both in terms of quantity and quality and is not likely to ensure domination of this species in future stands (Lygis et al. 2014). In time, respective silvicultural measures (cleanings, pre-commercial fellings and additional planting) may increase the proportion of valuable tree species (noble hardwoods, conifers) in the regenerated stands thus achieving good productivity and sanitary condition potential, although fraction of *F. excelsior* in the future stands will most likely be only negligible.

Recommendations for selection of common ash genotypes resistant to *H. fraxineus* and their use for establishment of resistant seed orchards in Lithuania have been prepared by Pliūra (2011, 2012). Recommendations for selection of *F. excelsior* genotypes resistant to *H. fraxineus* propose multi-step procedures for selection of best populations, families in best populations and individuals in best families in progeny trials (Pliūra 2011). The main steps of these procedures are presented in the next chapter. Recommendations for establishment of resistant seed orchards (Pliūra 2012) describe principles and approaches for selecting locations for seed orchard establishment in different provenance regions, choosing optimum plantation size, site selection and preparation, blocking and mixing of clones, tree spacing, planting, tending, genetic roguing and seed collection. These plantations should be considered as multifunctional and may serve not only as second-generation seed orchards for producing improved seeds, but also as clonal trials, tree breeding populations for performing crossings of the selected genotypes, *ex situ* gene conservation populations, and bases for vegetative mass propagation of most resistant genotypes.

Breeding of dieback-resistant *F. excelsior*

In Lithuania, a national programme for breeding of main valuable tree species including *F. excelsior* has been launched in 2003. However, breeding of *F. excelsior* for resistance against *H. fraxineus* has not yet been emphasized at that time. Recently (in 2015), a new programme for breeding of main Lithuanian forest tree species including breeding of *F. excelsior* for resistance, has been launched. Ash conservation and breeding programme is coordinated by the Dept. of Forest Genetic Resources at the Lithuanian State Forest Service. In order to guarantee the most stable resistance in the selected trees, a selection procedure is based on the so-called 'Pyramid principle' which integrates two types (quantitative and qualitative) of the inheritance of resistance (McDonald and Linde 2002 a,b; Baniulis et al. 2008). Two parallel schemes of tree breeding for resistance have been proposed (Figure 6): i) scheme A - selection based on testing of half-sib progeny from randomly selected trees in Lithuanian and West European *F. excelsior* populations with consecutive testing of clonal progeny; and ii) scheme B - selection based on testing of clonal and half-sib progeny from putatively resistant trees selected in natural Lithuanian *F. excelsior* populations.

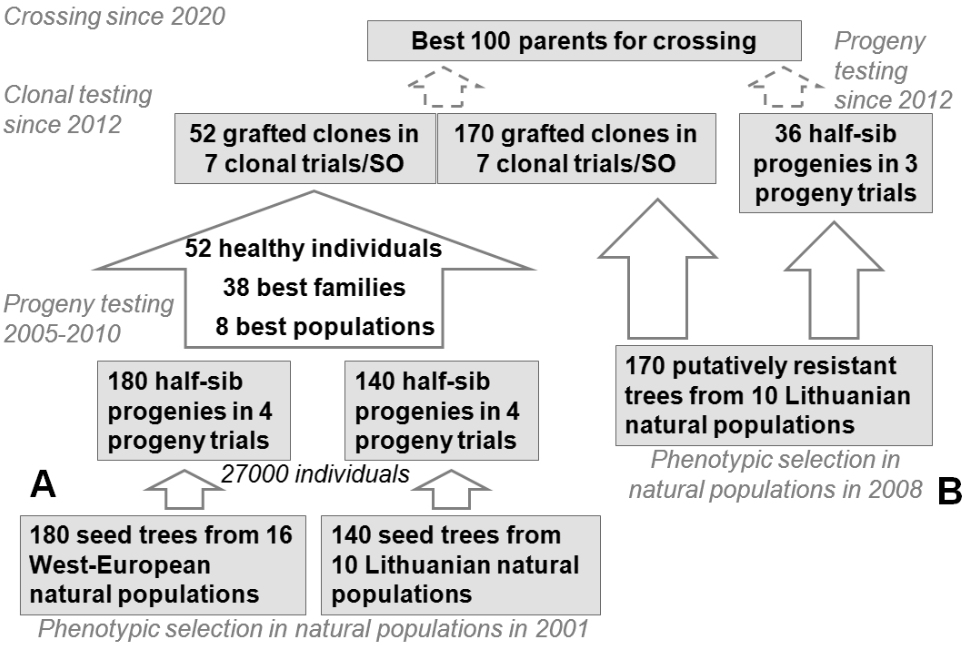


Figure 6 Two parallel schemes (A and B) used for breeding (selection and testing) of *Fraxinus excelsior* trees for resistance against dieback caused by *Hymenoscyphus fraxineus* in Lithuania

Scheme A. Based on testing of 320 *F. excelsior* half-sib families from 24 Lithuanian and West-European populations for eight years in progeny trials of 2005 year series (Figure 1), selection of least susceptible genotypes was carried out step-by-step on three levels: i) population, ii) family, and iii) individual (Pliūra et al. 2011). Following this step-by-step selection scheme, four best-performing Lithuanian and two foreign populations have been selected based on a complex breeding index value and on a proportion of individuals showing the best resistance/adaptability. In the second step, five to six half-sib families with the highest complex breeding BLUP indices (which includes scores of health condition, survival and height growth) and the highest proportion of individuals showing the best resistance were picked from each population selected in the first step. This enabled capturing additively inherited resistance. In the third step, two to three externally healthy individuals whose height had exceeded the trial mean were picked from the selected families, thus capturing monogenetically as well as poligenetically inherited individual resistance determined by gene interaction (additive, epistasis, dominance, pleiotropy, etc.) (Pliūra et al. 2011). In order to increase genetic gain in height and genetic variation, 11 individuals were picked among the tallest individuals growing in other tested populations in addition to the main step-by-step selection scheme. In addition, five control individuals of average resistance were selected from the best-performing populations selected based on complex breeding value indexes (Pliūra et al. 2011): from three Lithuanian (Kėdainiai, Šakiai and Žeimelis) and two foreign - Czech (Rabstejn) and German (Farchau) populations. As a result of this complex selection scheme, a total of fifty superior individuals have been selected for establishment of resistant seed orchards of second generation over all provenance regions of Lithuania. Grafting of the selected ash individuals was performed in 2010, and using this grafted material six seed orchards (covering a total area of 9.8 ha) and a clonal archive (2.8 ha) have been established in 2012 in three provenance regions of *F. excelsior* (Figure 1). The established seed orchards also serve as clonal trials and constitute breeding populations of *F. excelsior* based on Multiple Population Breeding System (MPBS) principles for long-term breeding for resistance. By 2020 it is planned to select the most resistant ash clones in each breeding population and to perform artificial crossings using a double-pair mating aiming at producing full-sib progeny and initiating new progeny testing cycle (integrating full-sib and clonal progeny testing).

Scheme B. Parallel to the selection based on the half-sib progeny testing, a total of 167 *F. excelsior* trees of good health condition have been phenotypically selected in natural populations severely affected by ash dieback. The scion shoots have been collected from these trees and used for grafting. The grafted seedlings were planted in the same clonal archive and in six seed orchards-clonal trials of 2012 year series for further testing (Figure 1). Seeds, where available, were collected for raising of half-sib progenies for progeny testing. In total, 36 half-sib families from three populations have been planted in three progeny trials (Figure 1). Selections from clonal and half-sib progeny trials will be included into MPBS breeding populations to be created under Scheme B.

Further research needs

Facing current ash dieback epidemic and aiming to apply proper stand management strategies it is of critical importance to continuously monitor sanitary condition of and natural regeneration in *F. excelsior* stands. Although some research is already done on natural forest regeneration in *F. excelsior* stands devastated by ash dieback disease and subjected to sanitary clear-fellings (Lygis et al. 2014), it would be very interesting to investigate current situation in the same sites also including some new clear-cut sites that meet the same criteria of selection. Studies of natural ash regeneration potential may also be expanded into uncut *F. excelsior* stands least damaged by the dieback. It is widely accepted that genetic diversity is crucial for disease resistance in forest tree populations (Burdon 2001). Revealing genetic diversity in and structure of the naturally regenerating ash populations (primarily considering *F. excelsior* individuals exhibiting low susceptibility to the dieback) would allow answering question about their future sustainability, i.e. first of all - if they meet a criterion of the effective population size. Moreover, it would be interesting to study natural forest succession in dieback-affected but not further managed *F. excelsior* stands where no felling of trees was done, and where stand development is continuously monitored on a regular basis.

Aiming to increase efficacy of *F. excelsior* selection and breeding for resistance against ash dieback there is a pressing need to find heritable tree traits or genetic markers associated with its susceptibility to *H. fraxineus* thus enabling straightforward selection of resistant genotypes.

Concluding remarks

Since the beginning of ash dieback epidemic in Lithuania in 1996, health condition of *F. excelsior* stands has catastrophically deteriorated leading to a massive tree mortality and consequent intensive sanitary fellings. Over past 20 years of the ongoing epidemic the country has lost more than a half of its forest stands where *F. excelsior* was a dominant tree species, and health condition of the remaining ash stands is still continuously deteriorating. According to data collected from permanent forest monitoring plots, about 9% of *F. excelsior* trees die out every year. Self-regeneration of *F. excelsior* in clear-cut areas is very poor both in terms of quantity and quality (health condition); here, ash is largely outnumbered by fast-growing birch, grey alder and aspen which will likely lead to only a minor (if any) fraction of ash in species composition of naturally regenerated forest stands.

Together with damages caused by *H. fraxineus* to tree crowns, root rot caused by *Armillaria* spp. are mentioned as the main reasons of the current decline of *F. excelsior* stands in Lithuania. Local population of *H. fraxineus* shows high pathogenicity towards *F. excelsior* although virulence of different isolates varies substantially. The performed studies revealed high genotypic but low genetic (allelic) diversity and showed no genetic structure in Lithuanian population of the ash dieback pathogen.

Due to the ongoing dieback and continuous deterioration of ash health condition there is a big loss in areas used for conservation of Lithuanian *F. excelsior* genetic resources. Nevertheless, since 2005, several progeny and clonal trials of *F. excelsior* have been established aiming to raise resistant material suitable for restoration of damaged ash stands and further breeding. Data collected in those progeny and clonal trials revealed substantial genetic variation and heritability of susceptibility/resistance traits in *F. excelsior* and indicate a good potential of selection and breeding of dieback-resistant ash propagation material. The obtained results also clearly showed that introduction of foreign *F. excelsior* populations to restore Lithuanian ash stands may have a doubtful potential and should be considered with care. High genetic diversity of material accumulated and tested in the field trials shows good potential for gene conservation *ex situ* and for breeding of *F. excelsior* for resistance against *H. fraxineus*.

Other studies revealed significant genotype by environment interaction for most of tree damage traits in *F. excelsior* (mortality rates, disease incidence and intensity of damage). Similar or even higher G × E variance component values were obtained for most of the damage traits compared to values obtained for tree height and bud flushing phenology. This indicates a presence of genetic variation in plasticity and reaction norms of ash clones across sites providing that resistance to disease of the most susceptible clones unequally depends on environmental conditions.

Detailed recommendations for management of dieback-affected ash stands have been prepared for Lithuanian forest owners and managers to propose options for thinnings and sanitary fellings in ash stands and retaining of most vigorous trees (possibly resistant to the dieback) as seed sources for natural regeneration and/or for breeding purposes. However, these recommendations are not always followed in practice or implemented measures are not always effective due to extremely high ash mortality rates and intensive deterioration of its health condition.

As options of effective management of the devastated stands and means for disease control are still quite limited, the research focuses and in the future will focus on one of the most promising options to save ash - its selection and breeding for resistance against *H. fraxineus*. Aiming to create a good basis for fulfilment of this task, recommendations for selection of *F. excelsior* genotypes resistant to *H. fraxineus* and their use for establishment of resistant seed orchards have been prepared, and a new national programme for breeding of main forest tree species including breeding of *F. excelsior* for resistance, has been launched.

Acknowledgements

The authors are grateful to the European Cooperation in the Field of Scientific and Technical Research (COST) Programme Action No. FP1103 ‘FRAXBACK’, Lithuanian long-term research programme ‘Sustainable Forestry and Global Changes’, Research group project ‘UOSIS’ No. MIP-040/2012 (2012-2014) and to National Programme project 'MIŠKOEKOKAITA' No. SIT-4/2015 (2015-2018) funded by Research Council of Lithuania.

References

Anonymous. 2001. Lietuvos miškų ūkio statistika. Lithuanian Statistical Yearbook of Forestry. L.R. Aplinkos ministerija, Valstybinė miškų tarnyba, Lututė, Kaunas [in Lithuanian and English].

Anonymous. 2015. Lietuvos miškų ūkio statistika. Lithuanian statistical yearbook of forestry. L.R. Aplinkos ministerija, Valstybinė miškų tarnyba. Lututė, Kaunas [in Lithuanian and English].

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.

Baniulis, D., Gelvonauskienė, D., Gelvonauskis, B., Rugienius, R., Bendokas, V., Stanys, V. 2008. Atsparumo rauplėms genų tyrimas ir molekulinių metodų taikymas obelų selekcijoje [Studies of scab resistance genes and application of molecular methods in breeding of apple trees]. *Sodininkystė ir daržininkystė* 27(3): 61-75. [in Lithuanian].

Burdon, R.D. 2001. Genetic diversity and disease resistance: some considerations for research, breeding, and deployment. *Canadian Journal of Forest Research* 31: 596-606.

Burokiene, D., Prospero, S., Jung, E., Marciulyniene, D., Moosbrugger, K., Norkute, G., Rigling, D., Lygis, V., Schoebel, C.N. 2015. Genetic population structure of the invasive ash dieback pathogen *Hymenoscyphus fraxineus* in its expanding range. *Biological Invasions* 17(9): 2743-2756.

Dobrowolska, D., Hein, S., Oosterbaan, A., Wagner, S., Clark, J., Skovsgaard, J.P. 2011. A review of European ash (*Fraxinus excelsior* L.): implications for silviculture. *Forestry* 84: 133-148.

Douglas, G.C., Pliura, A., Dufour, J. et al. 2013. Common Ash (*Fraxinus excelsior* L.). In: L. Paques (Editor) Forest Tree Breeding in Europe: Current State-of-the-Art and Perspectives Series: Managing Forest Ecosystems (Book 25). Springer, Dordrecht, pp.403-462.

FRAXIGEN. 2005. Ash species in Europe: biological characteristics and practical guidelines for sustainable use. Oxford Forestry Institute, University of Oxford, Oxford.

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V., Sieber, T.N. 2014. *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. *Molecular Plant Pathology* 15(1): 5-21.

Gustienė, A. 2010. Grybo *Chalara fraxinea* reikšmė uosių džiūties procese [Importance of fungus *Chalara fraxinea* in the process of ash dieback]. *Mūsų girios* [*Our Forests*] 11:18–19.

Juodvalkis, A. 2003. Ūkininkavimo pažeistuose uosynuose rekomendacijos [Recommendations for management of damaged ash stands]. Ministry of Environment of the Republic of Lithuania, Vilnius 11 pp. (ISBN 9955-539-14-3)

Juodvalkis, A. and Vasiliauskas, A. 2002. Lietuvos uosynų džiūvimo apimtys ir jas lemiantys veiksniai [The extent and possible causes of dieback of ash stands in Lithuania]. *LŽŪU Mokslo Darbai. Biomedicinos Mokslai* 56:17-22 [in Lithuanian with English summary].

Karpavičius, J., Vitas, A. 2006. Influence of environmental and climatic factors on the radial growth of European ash (*Fraxinus excelsior* L.). *Ekologija* 1: 1-9.

Kirisits, T., Freinschlag, C. 2012. Ash dieback caused by *Hymenoscyphus pseudoalbidus* in a seed plantation of *Fraxinus excelsior* in Austria. *Journal of Agricultural Extension and Rural Development* 4(9): 184-191.

Kowalski, T. 2006. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland. *Forest Pathology* 36(4): 264-270.

Lobo, A., McKinney, L.V., Hansen, J. K., Kjær, E.D., Nielsen L.R. 2015. Genetic variation in dieback resistance in *Fraxinus excelsior* confirmed by progeny inoculation assay. *Forest Pathology* 45(5): 379-387.

Lygis, V., Bakys, R., Gustiene, A., Burokiene, D., Matelis, A., Vasaitis, R. 2014. Forest self-regeneration following clear-felling of dieback-affected *Fraxinus excelsior*: focus on ash. *European Journal of Forest research* 133: 501–510.

Lygis, V., Vasiliauskas, R., Larsson, K-H., Stenlid, J. 2005. Wood-inhabiting fungi in stems of *Fraxinus excelsior* in declining ash stands of northern Lithuania, with particular reference to *Armillaria cepistipes*. *Scandinavian Journal of Forest Research* 20: 337-346.

Marčiulynienė, D., Lygis, V., Pliūra, A., Krivickaitė, R., Dabkevičius, Z. 2015. Ankstyvas paprastojo uosio šeimų jautrumo džiūčiai testavimas panaudojant dirbtinį apkrėtimą *Hymenoscyphus fraxineus* [Early testing of common ash families for susceptibility to dieback using artificial inoculation with *Hymenoscyphus fraxineus*] *Miškininkystė* 1(77): 7-19. (in Lithuanian with English summary).

McDonald, B.A., Linde, C. 2002a. Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology* 40: 349–379.

McDonald, B.A., Linde, C. 2002b. The population genetics of plant pathogens and breeding strategies for durable resistance. *Euphytica* 124(2): 163–180.

McKinney, L.V., Nielsen, L.R., Hansen, J.K., Kjær, E.D. 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (Oleraceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. *Heredity* 106: 788-797.

McKinney, L.V., Thomsen, I.M., Kjær, E.D., Nielsen, L.R. 2012. Genetic resistance to *Hymenoscyphus pseudoalbidus* limits fungal growth and symptom occurrence in *Fraxinus excelsior. Forest Pathology* 42: 69-74.

Narbutas, K. 1975. Lietuvos TSR uosynų tipai ir struktūra bei priemonės jiems atkurti ir produktyvumui pakelti [Types of ash stands in Lithuanian SSR and measures for their restoration and productivity improvement]. Dissertation, Lithuanian Forest Research Institute, Kaunas. [in Lithuanian].

Olrik, D.C., Kjaer, E.D., Ditlevsen, B. 2007. Klonforskelle i angreb af asketoptørre [Clonal variation in ash dieback]. *Skoven* 39: 522-525. [in Danish].

Ozolinčius, R. 2002. Uosynų džiūvimo hipotezės [Hypotheses for ash stand decline]. *Mūsų Girios* [*Our Forests*] 7: 2-4. [in Lithuanian].

Padaiga, V. 2002. Dar viena uosynų žūties hipotezė [One more hypothesis for ash stand decline]. *Mūsų Girios* [*Our Forests*] 10: 2-3. [in Lithuanian].

Pliūra, A. 1999. European long-term gene conservation strategies: Ash (*Fraxinus* spp). Proceedings of the 3rd EUFORGEN Noble Hardwoods Meeting, July 13–16, 1999, Sagadi, Estonia, p. 8-20.

Pliūra, A. 2011. Paprastojo uosio atsparių ligoms genotipų atranka ir naudojimas rezistencinėmis sėklinėms plantacijoms veisti [Selection of common ash genotypes resistant to diseases and their use for establishment of resistant seed orchards]. Naujausios rekomendacijos žemės ir miškų ūkiui – 2011 [New Recommendations for Agriculture and Forestry - 2011]. Lietuvos agrarinių ir miškų mokslų centras. Akademija, Kaunas, pp. 47–49 (ISSN 2029-7548) [in Lithuanian].

Pliūra, A. 2012. Paprastojo uosio rezistencinių sėklinių plantacijų veisimas. [Establishment of disease-resistant common ash seed orchards]. Naujausios rekomendacijos žemės ir miškų ūkiui – 2012 [New Recommendations for Agriculture and Forestry - 2012]. Lietuvos agrarinių ir miškų mokslų centras. Akademija, Kaunas, pp. 44–47 (ISSN 2029-7548) [in Lithuanian].

Pliūra, A., Baliuckas, V. 2007. Genetic variation in adaptive traits of progenies of Lithuanian and western European populations of *Fraxinus excelsior* L. *Baltic Forestry* 13(1): 28-38.

Pliūra, A., Heuertz, M. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for Common ash (*Fraxinus excelsior*). International Plant Genetic Resources Institute, Rome, Italy, 6 p.

Pliura, A., Lygis, V., Marciulyniene, D., Suchockas, V., Bakys, R. 2016. Genetic variation in susceptibility of *Fraxinus excelsior* half-sib families to *Hymenoscyphus fraxineus* following simulated spring frost and summer drought treatments. *iForest - Biogeosciences and Forestry* 8: 12-22.

Pliūra, A., Lygis, V., Suchockas, V., Bartkevičius, 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(1): 17–34

Pliūra, A., Marčiulynienė, D., Bakys, R., Suchockas, V. 2014. Dynamics of genetic resistance to *Hymenoscyphus pseudoalbidus* in juvenile *Fraxinus excelsior* clones. *Baltic Forestry* 20(1): 10-27.

Riepšas, E. 2009. Pažeistų uosynų atsikūrimo/atkūrimo būklė ir taikytinos priemonės [Regeneration of damaged common ash stands and recommended measures]. *Mūsų girios* [Our Forests] 10: 18-19. [in Lithuanian].

Skuodienė, L., Grybauskas, K., Palionis, V., Maslinskas, R. 2003. The health condition of ash stands and possible reasons of their dieback. *Miškininkystė* 54: 86-96. [In Lithuanian with English abstract].

Stener, L.-G. 2007. Studie av klonskillnader i känslighet för askskottsjuka [A study on clonal variation in susceptibility to ash dieback]. Arbetsrapport från Skogforsk 648, Uppsala, Sweden, [in Swedish].

Stener, L.-G. 2013. Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* L. in southern Sweden. *Scandinavian Journal of Forest Research* 28, 3: 205-216.

Tapper, P.-G. 1993. The replacement of *Alnus glutinosa* by *Fraxinus excelsior* during succession related to regenerative differences. *Ecography* 16(3): 212-218.

Verbyla V., Brukas A., Kairiūkštis L. (ed.) 2003. Lietuvos miškų metraštis XX a. [The chronicle of Lithuanian forests, XX century]. L.R. Aplinkos ministerija, Vilnius. 631 pp. [in Lithuanian with English summary].

Wagner, S. 1990. Zu: „Vereschung – Problem oder Chance“? [To: “Fraxinization - a problem or an opportunity”?]. *Allgemeine Forstzeitschrift* 32: 806-807. [in German].

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 166 – 175.

Ash dieback in Norway – current situation

I. BØRJA*, V. TIMMERMANN, A. M. HIETALA, M. M. TOLLEFSRUD, N. E. NAGY, A. VIVIAN-SMITH, H. CROSS, J. H. SØNSTEBØ, T. MYKING, H. SOLHEIM

NIBIO (Norwegian Institute of Bioeconomy Research), P.O. Box 115, NO-1431 Ås, Norway
** Corresponding author: isabella.borja@nibio.no*

Abstract

In Norway the common ash (*Fraxinus excelsior* L.) has its northernmost distribution in Europe. It grows along the coastal range as small fragmented populations. The first occurrence of ash dieback caused by *Hymenoscyphus fraxineus* in Norway was reported in 2008. At that time, the disease had already spread through large areas of southern and south-eastern parts of Norway. Since then the disease continued spreading with a speed of about 50-60 km per year along the western coastal range.

To monitor the disease development over time, we established eight permanent monitoring plots in south-eastern and western Norway in 2009 and 2012, respectively. In all plots tree mortality was high, especially among the youngest trees in south-eastern Norway. The extent of crown damage has continually increased in all diameter classes for both regions. In 2009, 76.8 % of all trees on the five monitoring plots in south-eastern Norway were considered to be healthy or slightly damaged, and only 8.9 % to be severely damaged. In 2015, 51.7 % were dead, 13.5 % severely damaged and only 25.7 % remained healthy or slightly damaged.

To assess the infection pressure and spore dispersal patterns of the pathogen, we used a Burkard volumetric spore sampler placed in an infested ash stand in southern Norway. We examined the airborne ascospores of *H. fraxineus and H. albidus* captured on the sampling tape microscopically and with real-time PCR assays specific to these fungi. We detected very few ascospores of *H. albidus*, whereas ascospores of *H. fraxineus* dominated throughout entire sampling periods of 2009, 2010 and 2011. Spore discharge occurred mainly between the hours of 5 and 8 a.m., though the distinctive sporulation had yearly variation between 5-7 a.m. We observed the same diurnal pattern throughout the entire sampling period, with a seasonal peak in spore liberation between mid-July and mid-August, after which the number of ascospores decreased substantially. Similar diurnal patterns were observed throughout the sampling period except that after mid-August the number of trapped ascospores substantially decreased.

To compare the genetic pattern of common ash in the northern and central ranges of Europe we analyzed the Norwegian samples together with available samples from central Europe by using chloroplast and nuclear microsatellite markers. We found that the northern range of common ash was colonized via a single migration route that originated in eastern or south-eastern Europe with little influence originating from other southern or western European refugia. In the northern range margins, genetic diversity decreased and population differentiation increased, coherent with a post-glacial colonization history characterized by founder events and population fluctuations. Based on our findings we discuss the future management and conservational implications.

Keywords: ascospore production, epidemiology, genetic structure, *Hymenoscyphus fraxineus*, invasive alien pathogens, real-time PCR, sporulation

Introduction

Europe is currently experiencing a rapid spread of the invasive fungal pathogen *Hymenoscyphus fraxineus* causing a serious dieback of common ash trees (*Fraxinus excelsior* L.). The first observation of ash dieback by this pathogen was recorded in Poland in 1990. Since then the disease progressed swiftly across the European continent and currently it is established throughout most of Europe except for its southernmost parts.

Norway has the northernmost population of common ash in Europe. The ash is growing along Norway's coastal range from the southeast through south to the western part, up to mid-Norway (63.5° N). The ash dieback in Norway was first confirmed in 2008. However, because of the finds of older symptomatic necrotic tissue, the disease could have been present here already in 2006, or even earlier.

Because the rapid spread, high infection pressure and high susceptibility of common ash are the key factors in the success of the pathogen, we focused in our research on the monitoring of the pathogen spread, disease development and genetic structure of ash trees. Here we summarize our latest results on the escalation of ash dieback disease and the current situation in Norway.

Monitoring of geographic spread in Norway

To monitor the geographic occurrence and the rate of ash dieback spread we surveyed the annual spread of the disease from 2009 onwards along the coast of Norway. At each locality we visually evaluated the health status of ash trees and took samples where the first symptoms appeared.

In 2008 the ash dieback was reported for the first time from large areas of southern and south-eastern part of Norway. In 2009 the disease spread to municipality of south Rogaland and one year later it was found also in the north Rogaland. In 2012 the disease was reported from several new localities in the county of Sogn and Fjordane. The rate of disease spread was about 30 km per year initially. Later, when the disease spread further north along the western coast the rate increased and became more variable, in average about 60-70 km per year (Solheim, unpublished data). Because *Hymenoscyphus fraxineus* spreads mostly by air through ascospores, we hypothesized that the spread to neighbouring ash stands over shorter distances is mediated by airborne ascospores, whereas spread over long distances probably happens by import or transfer of diseased plants. Molecular data from genome-wide high through-put sequencing of *Hymenoscyphus fraxineus* isolates from Norway however shows that the genetic structure throughout Norway and the rest of Europe is extremely uniform, potentially as the result of a small founder population. This data provides an alternative hypothesis in that introductions to new localities throughout Norway are due mostly to ascospore spread by meteorological phenomena (Sønstebø, Vivian-Smith et al, unpublished data).

Monitoring of ash dieback on permanent plots in Norway

To study disease progression in individual ash trees of varying age and differences in disease development between regions with different disease history, we established several permanent monitoring plots in two regions; five plots in south-eastern Norway and three plots in western Norway.

We followed the disease progression in individual ash trees by assessing defoliation and other crown damage parameters in ash trees of different diameter classes in south-eastern Norway from 2009 and in western Norway from 2012 (**Fel! Hittar inte referenskälla.**). All plots are situated in ash dominated forest stands with an area of 100–1000 m^2, while stand area is ranging from a few 100 m^2 to almost 30 ha. On four plots showing distinct age classes we selected 10 large, dominant trees with varying degree of crown damage and 40 healthy appearing young trees, 50 trees in all. On the remaining four plots with more even age distribution, we selected 40 trees in total (Timmermann et al. 2013).

We assessed the crown condition and damage symptoms visually on individual trees once a year on each plot following standardized methods developed by ICP Forests (UNECE 2010). To group the trees into damage classes (Table 1), we used defoliation scores, considered to be the most important damage variable. We measured growth every five years, and grouped the trees in three diameter classes according to their diameter at breast height (DBH) in 2014: Juvenile or small trees (DBH <5 cm), intermediate, future crop trees (DBH 5–12.5 cm) and large, dominant trees (DBH >12.5 cm). Although tree heights are overlapping between diameter classes, diameter can reflect age to a certain degree.

Table 1 Grouped defoliation classes and degree of defoliation.

Grouped defoliation classes
Healthy trees (0-10 % defoliation)
Slightly damaged (11-25 % defoliation)
Moderately damaged (26-50 % defoliation)
Severely damaged (51-99 % defoliation)
Dead trees (100 % defoliation)

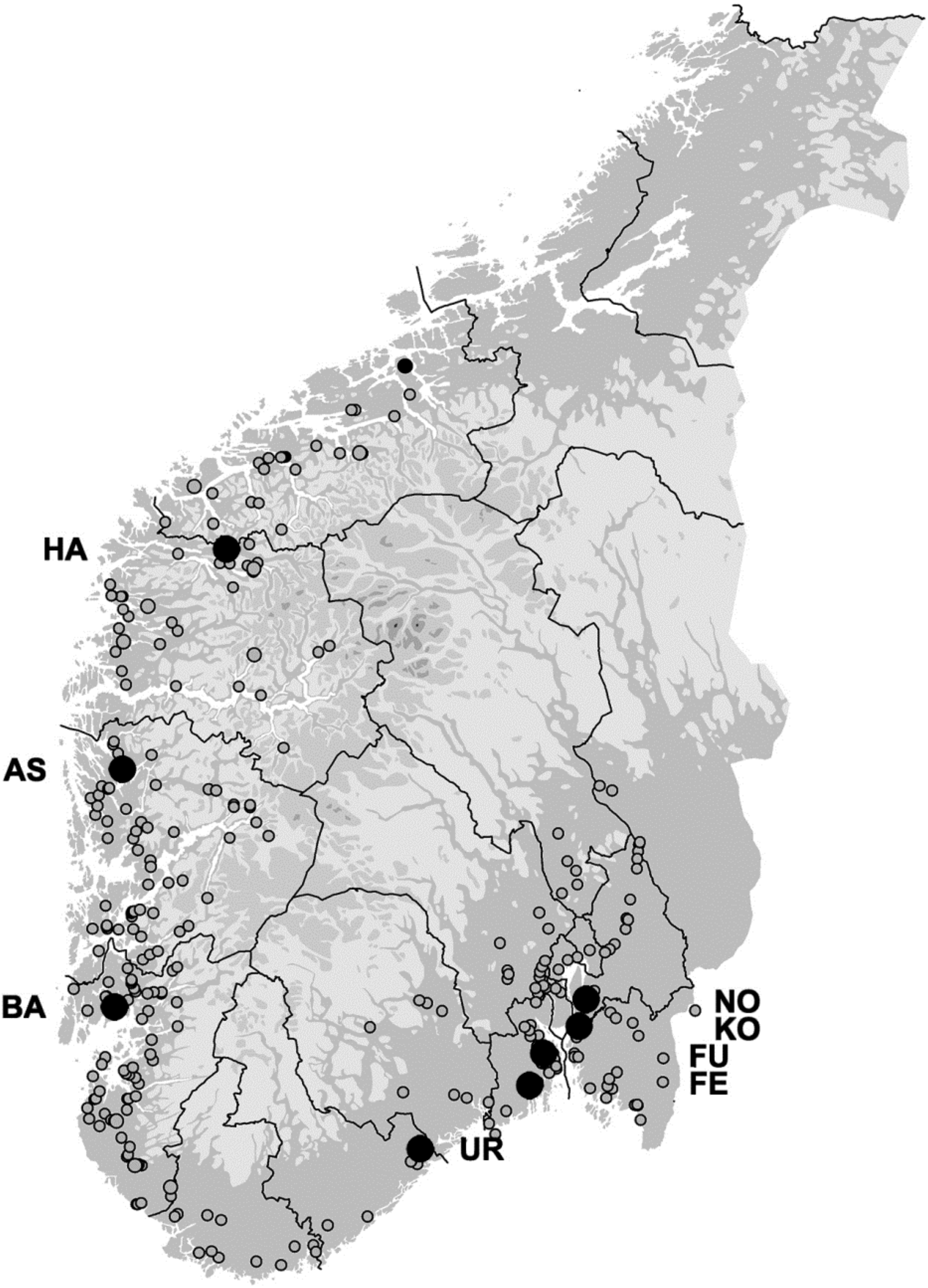


Figure 1 Location of monitoring plots (large black dots) and observations of ash dieback confirmed by either isolation or sequencing in Norway 2008–2015 (small grey dots, the northernmost location in black).

South-eastern Norway

<u>All diameter classes:</u> In 2009, 76.8 % of all trees in all diameter classes on the five monitoring plots in south-eastern Norway were considered to be healthy or slightly damaged, and only 8.9 % to be severely damaged. In

2015, 51.7 % were dead, 13.5 % severely damaged and only 25.7 % still healthy or slightly damaged (**Fel! Hittar inte referenskälla.**a, Timmermann et al. 2016).

Juvenile trees: In 2009, 86.1 % of the juvenile trees on the monitoring plots in south-eastern Norway appeared healthy or only slightly damaged and 4.6 % were severely damaged. In 2015, the proportion of healthy or slightly damaged juvenile trees was reduced to only 11.7 %, while 85.6 % of the young trees were severely damaged or dead.

Future crop trees: Although disease development has not been as rapid for the intermediate as for the juvenile trees in south-eastern Norway, also in this group there has been a considerable increase in the number of severely damaged and dead trees from 2009 to 2015. Based on defoliation assessments, 70 % of the trees in this diameter class were healthy or slightly damaged, and 12.5 % severely damaged in 2009. In 2015, 52.2 % of these future crop trees were either dead or severely damaged, and only 29.9 % still healthy or slightly damaged.

Dominant trees: Defoliation within the group of large, dominant trees had less increase relative to that observed for the younger trees located in south-eastern Norway from 2009 to 2015. The proportion of healthy or slightly damaged trees decreased only by 9.5 % from 2009 to 2015. Nonetheless, the proportion of severely damaged trees increased from 16.7 % to 25 % in the same period, and 13.5 % of the dominant trees were dead in 2015.

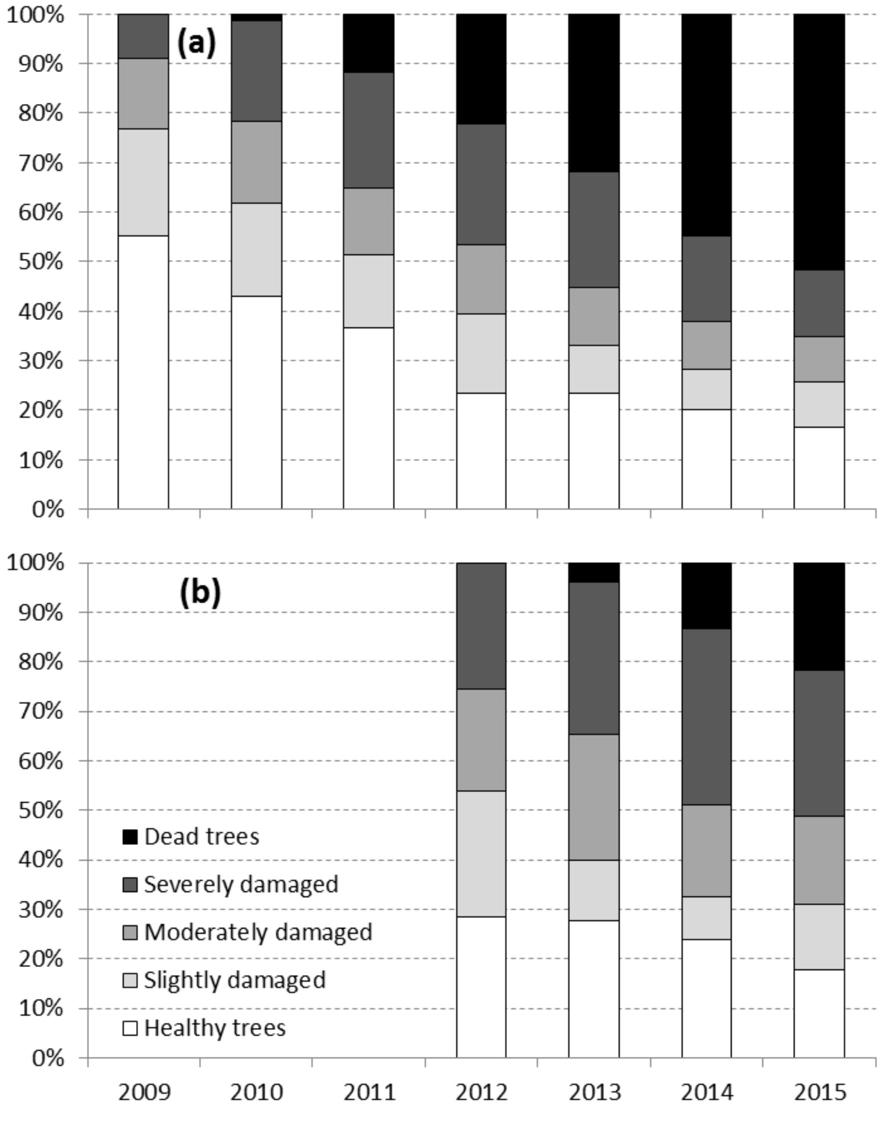


Figure 2 Trees (all diameter classes) grouped in defoliation classes in south-eastern plots 2009-2015 (a) and in western plots 2012-2015 (b).

Western Norway

In western Norway, clear symptoms of ash dieback and respective damage have been observed since 2013 (**Fel! Hittar inte referenskälla.**b) on two of the monitoring plots, reflecting the same trends that were found in south-eastern Norway with a high mortality rate amongst the youngest trees and only a slight worsening for the oldest trees. In the third, northernmost plot still no clear signs of ash dieback were detected, except for some leaf wilting on a few trees in 2015, although the disease has spread to that area and even farther north.

Monitoring of sporulation-patterns

The airborne ascospores of *H. fraxineus* cause the primary infection of ash trees and facilitate dispersal of the fungus in Europe. To study the diurnal and seasonal variation in ascospore emission by *H. fraxineus*, we sampled spores during three years, in the periods 29.07.–23.09.2009 (Timmermann et al. 2011), 02.07.–07.10.2010 (Solheim et al. 2011) and 04.07.–25.07.2011 (Hietala et al. 2013).

We sampled spores by using a solar power-driven Burkard 7-day volumetric spore sampler (Burkard Scientific, Uxbridge, UK) installed at ground level in a diseased stand of pure common ash located 30 km south of Oslo (Ås municipality, 59° 40' 44" N, 10° 46' 31" E, 100 m a.s.l.). At an air throughput of 10 l/min, airborne particles were collected onto an adhesive-coated, transparent Melinex tape, fastened to a revolving drum which moved past the air intake of the spore sampler at a rate of 2 mm per hour. With the collection tape being changed weekly we documented a continuous temporal record of airborne propagules.

We then divided tape samples, representing randomly chosen days during sampling period, into 48-mm-long segments, each corresponding to 24 hours of sampling. We stained each tape segment with lactofuchsin and analysed the images at 200x magnification along a longitudinal segment using a light microscope with a digital camera mounted. We recorded the amount of ascospores with morphological characteristics similar to *H. albidus/H. fraxineus* as described by Kowalski & Holdenrieder (2009).

Because ascospores of *H. albidus* and *H. fraxineus* are morphologically similar, and thus difficult to distinguish by microscopy (Fig.3), we used in addition species-specific real-time PCR assays on DNA extracted from spore tape samples (Hietala et al. 2013) to examine the relative proportions of the two species during the sporulation season. As reference, we used weather data from a meteorological station located in an orchard 1500 m south of the studied ash stand (http://lmt.nibio.no/weatherstations).

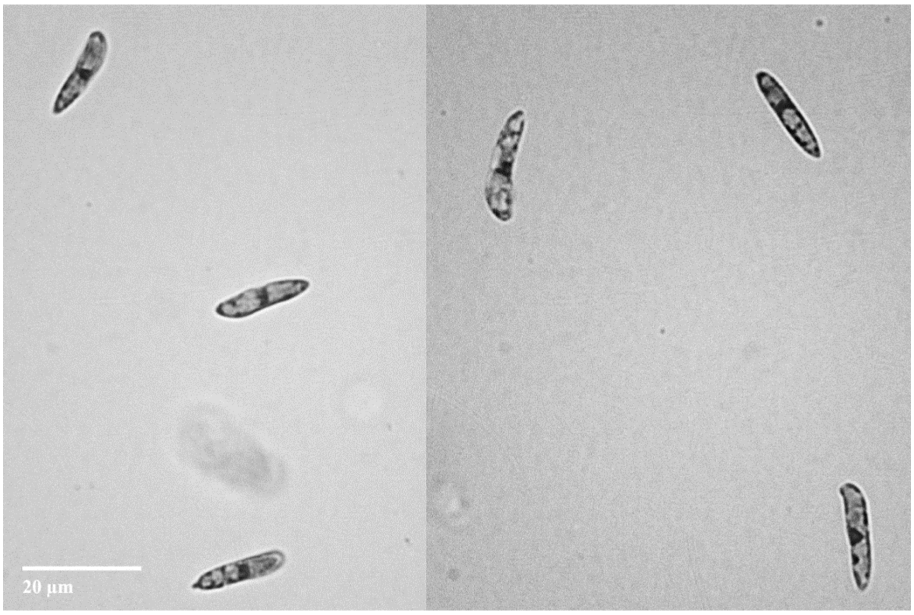


Figure 3 Microscope (x400) images of lactofuchsin stained ascospores. Left: *Hymenoscyphus albidus* (spore length 15.5-16.5 µm; Bergen, 17.8.2009). Right: *H. fraxineus* (spore length 18-19 µm; Sandefjord, 11.8.2009). Photo: Volkmar Timmermann. Originally published as color illustration in Solheim et al. (2011).

We found that in 2009-2011 *H. albidus/fraxineus* -like apothecia were present in ash leaf litter between the 1st week of July and late September. In our experimental stand the number of *H. albidus/fraxineus* -like ascomata during the end of July could be as high as 8300 fruit bodies per square meter (Hietala et al. 2013). The fruit bodies typically formed throughout the former conductive tissue of the compound ash leaf. We detected ascospores of *H. albidus*/*H. fraxineus,* identified by spore morphology and size, during the entire sampling periods in 2009, 2010 and 2011.

Spore discharge occurred mainly between the hours of 5 and 8 a.m., though the distinctive sporulation peak had yearly variation between 5 to 7 a.m. ((Fig. 4). We counted upper maxima of 10,000 spores per 0.1 mm^2 of tape area which corresponds to a pathogen pressure of 4.7 million spores per cubic meter of sampled air, roughly equating to upper maxima of 2.8 million spores discharged per hour. Similar diurnal patterns were observed throughout the sampling period except that after mid-August the number of trapped ascospores substantially decreased.

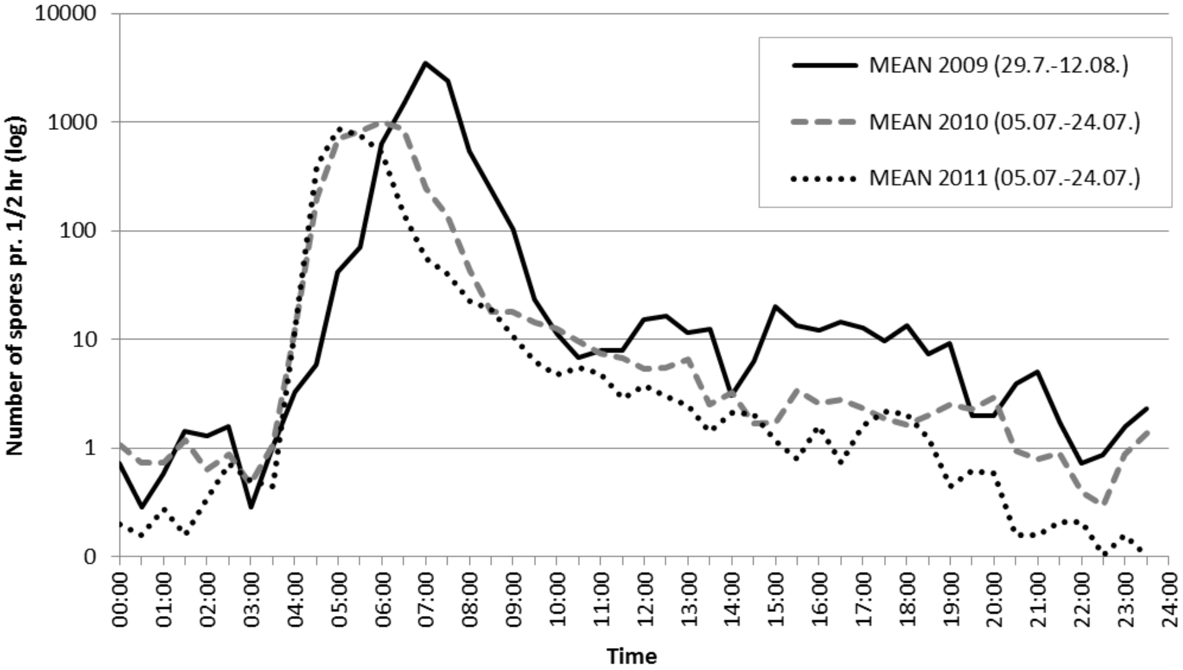


Figure 4 Diurnal variation in ascospore emission by Hymenoscyphus fraxineus in a diseased ash stand in Southern Norway in 2009–2011 analyzed by microscopy.

In 2010 and 2011, we noted a drastic increase in the number of airborne *Hymenoscyphus* ascospores in mid-July (Fig. 5). The period with high levels of these ascospores lasted until mid-August, after which the spore number declined rapidly to the low level seen in the beginning of July. Although the 2010 spring was colder than the spring of 2011, both years showed an increase in release of *Hymenoscyphus albidus*/*fraxineus*-like ascospores during the first two weeks of July. The maximum spore numbers were similar between the years and occurred during the warmest period of the summer between mid-July and mid-August.

There was in general a good correlation between the spore number datasets established by microscopy and *H. fraxineus* targeted real-time PCR. We detected the presence of *H. albidus* only on one day, 21.07.2010. The estimated ascospore number of the fungus was very low, 0.23% of the estimated ascospore number of *H. fraxineus* during that period.

Genetic structure of common ash in Norway

Common ash is a temperate tree species with a wide distribution across Europe. Its distribution ranges from the Mediterranean and the Caspian Sea in the south to mid-Norway (63.5° N) in the north, and from the Atlantic coast in the west into continental Russia in the east (Pliūra and Heuertz, 2003).

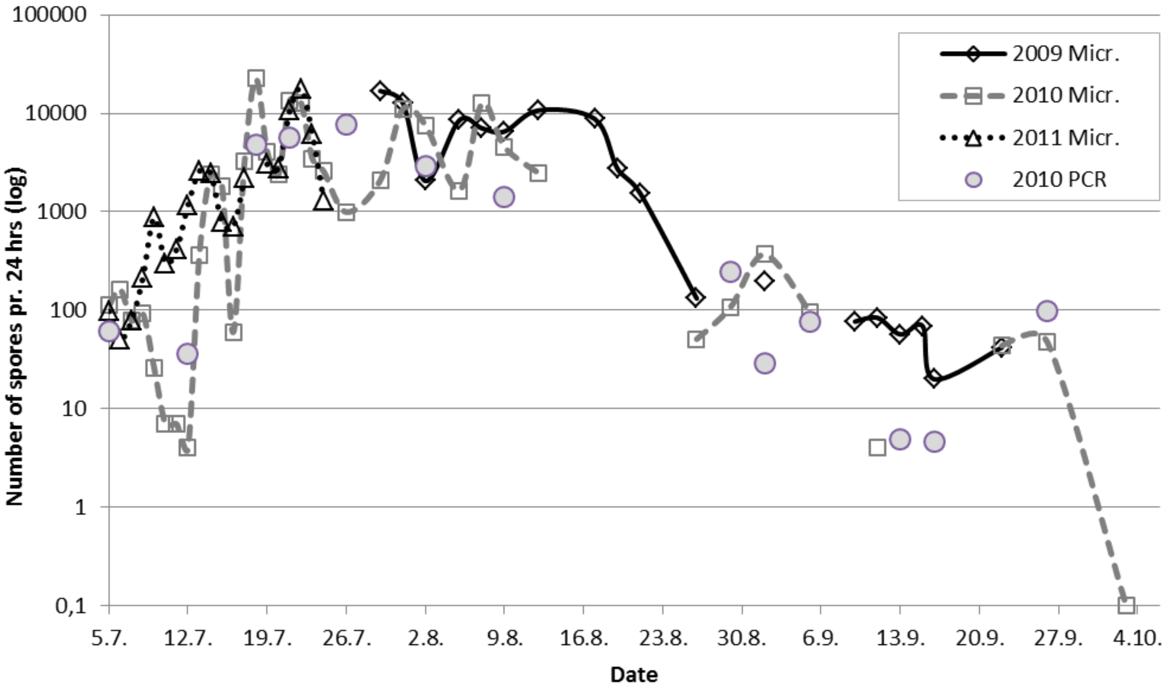


Figure 5 Seasonal variation in ascospore emission by *Hymenoscyphus fraxineus* in a diseased ash stand in Southern Norway in 2009–2011 analyzed by microscopy and real-time PCR (only in 2010).

Because of the rapid spread of the invasive pathogen *H. fraxineus*, knowledge on the host tree species population genetic structure is a prerequisite for risk assessment and effective disease management. To build a basis for management and conservation practices for northern common ash populations we investigated the population history and genetic structure of Norwegian common ash populations (Tollefsrud *et al*., Submitted). Although common ash has been the subject of several genetic studies (e.g. Heuertz *et al*., 2004*a*, *b*; Sutherland *et al*., 2010;Gerard et al. 2013), little information is available about the genetic diversity at its northern range margins.
To evaluate the genetic variation pattern of common ash in its northern range, we analyzed the Norwegian samples together with available samples from central Europe. We collected 17 populations (570 trees) of common ash throughout the Norwegian distribution range, 16 of the populations from nature reserves. We placed the Norwegian populations into a phylogeographical framework using maternally inherited plastid microsatellites using the same markers as Heuertz *et al*. 2004*a*. Furthermore we screened the samples with nuclear micorsatellites (Sutherland *et al*., 2010), to test specifically if the northern range margin populations of common ash in Norway exhibit lower genetic diversity, greater genetic divergence and higher rates of inbreeding than populations of the central European range, consistent with a leading edge colonization model.

We found, that all common ash populations from Norway showed the plastid DNA haplotype H01 (Tollefsrud et al. Submitted), which is characteristic to eastern and south-eastern European populations (Heuertz *et al*., 2004*a*). The gene pool structure of nuclear microsatellite data analyzed by using Bayesian clustering analysis additionally linked the northern range populations to eastern and south-eastern European populations. Both the plastid and the nuclear genetic data suggest that the northern range of common ash was colonized via a single migration route that originated in eastern or south-eastern Europe with little influence from other southern or western European refugia.

In the northern range of common ash in Norway, we detected a steep latitudinal decrease in both allelic richness and expected heterozygosity along with increased genetic differentiation among populations. Our nuclear microsatellite analysis moreover indicates that the northernmost population in Norway, Hindrum in Trøndelag, constitutes a separate genetic group with very low levels of genetic diversity. This genetic pattern of common ash in Norway is coherent with a leading edge colonization model (Hampe and Petit, 2005). In Norway, common ash is mainly restricted to a narrow nemoral forest belt along the coast, etched by fjords, valleys and high mountain ridges. Colonization of this coastal range potentially occurred in unidirectional mode through repeated establishments of small isolated populations from the leading colonizing front. The genetic peculiarity of the northernmost population, Hindrum in Trøndelag, is probably due to a founder effect (i.e. the population may have been established by a very small number of individuals from a larger population), because of recent long-distance colonization. Only two of the analyzed common ash populations in Norway had inbreeding coefficients significantly larger than zero, in agreement with other studies which have shown that common ash is predominantly outcrossing species (e.g. Bacles *et al*., 2005).

Management and conservation implications

Based on the available field data from several countries, McKinney *et al*. (2014) estimated that ~1-5% of the trees in native populations of common ash possess some form of resistance against the ash dieback pathogen. A low-intensity management approach, making use of this genetic variation, would be to initiate the preservation of individuals having good field performance under the natural infection pressure for future production and selection of less susceptible offspring (McKinney *et al*., 2014). Removal of diseased trees could be anticipated to promote breeding between robust individuals, while decreasing the local infection pressure, since infection by *H. fraxineus* is caused by ascospores that are released in abundance from fruit bodies growing on the overwintered ash leaf tissues (e.g. Timmermann *et al*. 2011; Hietala *et al*. 2013). Promotion and protection of natural regeneration (for detailed suggestions see e.g. Douglas *et al*., 2013) to meet the ash dieback-driven selection pressure would be applicable measure, even in nature reserves, and would increasingly be important in countries like Norway which lack breeding programs for common ash.

The genetic effects of ash dieback such as loss of allelic structure and increased biparental inbreeding may escalate more rapidly in the northern ranges where the smaller and fragmented populations tend to have lower allelic richness and are less interconnected through gene flow than within the central European populations. Allelic richness estimated from neutral markers has been shown to be a better indicator than expected heterozygosity for the adaptive potential of quantitative traits (Vilas *et al*., 2015), and introduction of new alleles via planting of healthy individuals into the populations could be one option to prevent loss of genetic diversity. At the same time, introduction of new alleles may break down local adaptation. For planting in Norwegian natural habitats where provenance trials are currently lacking, seedlings raised from local seed sources would be preferable. Evidence for local adaptation has been observed for common ash from continental Europe, where significant differences in survival and growth characteristics were detected among provenances from the Netherlands, Germany, Lithuania, Romania, Belgium and Switzerland (Douglas *et al*., 2013). Reciprocal transplant trials in England showed on the other hand no home site advantage for the tree species (Boshier and Stewart, 2005). Within Norway, local adaptation may be expected as environmental gradients follow latitudinal as well as coast-inland gradients.

For *ex situ* conservation of common ash in Norway, we collected in the autumn of 2015, seeds from 41 healthy common ash trees. The trees were in, or in the vicinity of six monitoring plots, in addition to the northernmost population in Norway, Hindrum inTrøndelag aiming to cover the total genetic diversity of common ash in Norway. The seed trees have been marked for future monitoring of health status. In 2016/2017, the seeds will be grown the first year in a greenhouse and phenological traits like time of leaf flushing, bud set and length of vegetation period will be recorded. Two year old ash seedlings will then be planted out for natural infection.

Conclusions

In Norway the geographical spread of ash dieback is rather fast but variable, in average more than 50 km per year, with newly infected locations detected every year and the infection range ever expanding. Juvenile trees with small DBH have higher mortality and are more severely damaged by the disease than larger trees since necroses on their stems may lead to dieback of the top or even the whole tree, while it takes several years and numerous necroses to kill major branches in old trees. Earlier studies conducted close to one of the monitoring plots in South-eastern Norway showed that during summer enormous amounts of ascospores are released by *Hymenoscyphus fraxineus* ascocarps growing on ash leaf litter (Timmermann et al. 2011, Hietala et al. 2013). It is therefore reasonable to assume that infection pressure for small, juvenile trees is much higher than for large, dominant trees that have their foliage 10 to 20 metres above ground. Juvenile trees are also more exposed to competition with other vegetation and will thus easier die off if they in addition are infected by ash dieback. Thus the ash dieback disease in Norway severely hampers the recruitment of young plants.

We showed that in a diseased ash forest mostly ascospores of *H. fraxineus* were present. The massive simultaneous spore discharge in the morning hours takes advantage of leaf wetness from morning dew to protect the ascospores against desiccation. Warm and moist conditions generally favour plant infection by foliar fungal pathogens, furthermore sporulation during summer months with a seasonal peak from mid-July to mid-August allows pathogen establishment in the current year leaves that are still fully nutritious and prior to competition posed by leaf senescence and soil-associated saprobes.

Populations of common ash at the northern range margins show loss of diversity and increased divergence, most probably as a consequence of a leading edge colonization process. Management practices to aid recruitment of healthy trees should be undertaken to prevent loss of genetic diversity due to selection pressure exerted by ash dieback.

Acknowledgements

We wish to thank Norwegian Research Council for financial support (project no 203822/E40), Norwegian Genetic Resource Centre and FRAXBACK COST action FP 1103.

References

Bacles CFE, Burczyk J, Lowe AJ, Ennos RA. 2005. Historical and contemporary mating patterns in remnant populations of the forest tree *Fraxinus excelsior* L. Evolution 59: 979-990.

Boshier D, Stewart J. 2005. How local is local? Identifying the scale of adaptive variation in ash (*Fraxinus excelsior* L.): results from the nursery. Forestry, 78: 135-143.

Douglas GC, Pliura A, Dufour A, et al. 2013. Common Ash (*Fraxinus excelsior*) In: Pâques LE, ed. Forest Tree Breeding in Europe: current state-of-the-art and perspectives.: Springer.

Gerard PR, Temunovic M, Sannier J, Bertolino P, Dufour J, Frascaria-Lacoste N, Fernandez-Manjarres JF. 2013. Chilled but not frosty: understanding the role of climate in the hybridization between the Mediterranean *Fraxinus angustifolia* Vahl and the temperate *Fraxinus excelsior* L. (Oleaceae) ash trees. Journal of Biogeography 40: 835-846.

Hampe A, Petit RJ. 2005. Conserving biodiversity under climate change: the rear edge matters. Ecology Letters 8: 461-467.

Heuertz M, Fineschi S, Anzidei M, et al. 2004a. Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe. Molecular Ecology 13: 3437-3452.

Heuertz M, Hausman JF, Hardy OJ, Vendramin GG, Frascaria-Lacoste N, Vekemans X. 2004b. Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southeastern European populations of the common ash (*Fraxinus excelsior* L.). Evolution 58: 976-988.

Hietala AM, Timmermann V, Børja I, Solheim H. 2013. The invasive ash dieback pathogen *Hymenoscyphus pseudoalbidus* exerts maximal infection pressure prior to the onset of host leaf senescence. Fungal Ecology 6: 302-308.

Kowalski T, Holdenrieder O, 2009. The teleomorph of *Chalara fraxinea*, the causal agent of ash dieback. Forest Pathology 39: 304–308.

McKinney LV, Nielsen LR, Collinge DB, Thomsen IM, Hansen JK, Kjaer ED. 2014. The ash dieback crisis: genetic variation in resistance can prove a long-term solution. Plant Pathology 63: 485-499.

McKinney LV, Thomsen IM, Kjær ED, Bengtsson SBK, Nielsen LR, 2012. Rapid invasion by an aggressive pathogenic fungus (*Hymenoscyphus pseudoalbidus*) replaces a native decomposer (*Hymenoscyphus albidus*): a case of local cryptic extinction? Fungal Ecology 5: 663-669.

Pliūra A, Heuertz M. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for common ash (*Fraxinus excelsior*). In: International Plant Genetic Resources Institute R, Italy, ed. *EUFORGEN Technical Guidelines for Genetic Conservation and Use*. http://www.euforgen.org/publications/publication/common-ash-emfraxinus-excelsiorem/: International Plant Genetic Resources Institute, Rome, Italy.

Solheim H, Timmermann V, Børja I, Hietala AM, 2011. En liten sekksporesopp, *Hymenoscyphus pseudoalbidus*, truer aska i Europa. [A small ascomycete, *Hymenoscyphus pseudoalbidus*, is a threat to common ash in Europe]. Agarica 30: 81-88. (in Norwegian with English summary)

Sutherland BG, Belaj A, Nier S *et al*. 2010. Molecular biodiversity and population structure in common ash (*Fraxinus excelsior* L.) in Britain: implications for conservation. Molecular Ecology 19: 2196-2211.

Timmermann V, Børja I, Hietala AM, Kirisits T, Solheim H. 2011. Ash dieback: pathogen spread and diurnal patterns of ascospore dispersal, with special emphasis on Norway. EPPO Bulletin 41: 14–20.

Timmermann V, Solheim H, Clarke N, Aas W, Andreassen K. 2013. Skogens helsetilstand i Norge. Resultater fra skogskadeovervåkingen i 2012 [The health state of the Norwegian forests. Results from the national forest damage monitoring 2012]. Rapport fra Skog og landskap 12/13: 32 pp. (in Norwegian with English summary)

Timmermann V, Andreassen K, Clarke, N, Nordbakken JF, Solheim H, Økland B, Aas W. 2016. Skogens helsetilstand i Norge. Resultater fra skogskadeovervåkingen i 2015 [The health state of the Norwegian forests. Results from the national forest damage monitoring 2015]. *In prep.*

Tollefsrud MM, Myking T, Sønstebø JH, Lygis V, Hietala A, Heuertz M. Genetic structure in the northern range margins of common ash, *Fraxinus excelsior* L. Submitted.

UNECE (United Nations Economic Commission for Europe) 2010. Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. Revised 2010. UNECE ICP Forests Programme Co-ordinating Centre, Hamburg. ISBN: 978-3-926301-03-1. [http://icp-forests.net/page/icp-forests-manual]

Vilas A, Perez-Figueroa A, Quesada H, Caballero A. 2015. Allelic diversity for neutral markers retains a higher adaptive potential for quantitative traits than expected heterozygosity. Molecular Ecology, 24: 4419-32.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 176 – 184.

Ash dieback in Poland – history of the phenomenon and possibilities of its limitation

W. GIL*[1], T. KOWALSKI[2], W. KRAJ[2], T. ZACHARA[1], J. ŁUKASZEWICZ[1], R. PALUCH[3], J. A. NOWAKOWSKA[4], T. OSZAKO[5]

[1] Dept. of Silviculture and Forest Tree Genetics, Forest Research Institute, Sękocin Stary, Braci Leśnej 3, PL05090 Raszyn, Poland, [2] Department of Forest Pathology, Mycology and Tree Physiology, Agricultural University in Cracow, Al. 29-Listopada 46, 31-425 Cracow, Poland, [3]Department of Natural Forests, Forest Research Institute, Park Dyrekcyjny 6, PL17230 Białowieża, Poland, [4]Molecular Biology Laboratory, Forest Research Institute, Sękocin Stary, Braci Leśnej 3, PL05090 Raszyn, Poland, [5]Dept. of Forest Protection, Forest Research Institute, Sękocin Stary, Braci Leśnej 3, PL05090 Raszyn, Poland
**corresponding author: GilW@ibles.waw.pl*

Abstract
The phenomenon of dying stands of ash was observed in Poland since 1993. The aim of the study was to assess the health condition of ash stands in the State Forests and to develop silviculture methods for subsequent phases of forest growth in stands with a significant share of ash, in order to minimize the negative consequences of ash dieback and to increase the durability and sustainability of these stands. In order to assess its scale, in 2004, we sent a questionnaire to all forest districts of the State Forests, operating on the surface of approx. 7.6 million hectares equaled 83% of the forest area. The article presents data on the extent and impact of ash dieback in Poland, genetic structure of Polish ash populations revealed by the DNA marker studies, genetic variability of Hymenoscyphus fraxineus in Poland and recommends silvicultural options to mitigate impact of the disease.

Introduction

Ash stands in Poland (with prevailing ash) occurred at the beginning of the twenty-first century, on an area of approximately 28.5 thousand ha - less than 1% of the total forest area of Poland (9.1 million ha) (Gil *et al*. 2006). On the most eutrophic habitats and wetland, where ash can be a dominant species or provide significant admixture its importance is the highest. The most common are the habitats of humid forests (more than 9.5 thousand ha), fresh forest (5,5 thousand ha), alder-ash stands (5.2 thousand ha) and riparian forests (2.3 thousand ha). The largest area of forest stands of ash appeared in Polish regions characterized by mild sub-Atlantic climate, especially in the south - western part of the country.

In Poland the growth of ash trees was not hampered by serious fungal or bacterial diseases (Grzywacz 1995). However, this situation fundamentally changed in the late twentieth century. In 1992, there were first signs of severe dieback of ash in the north-eastern Poland (Stocki 2001). In the following years this process has spread to other areas of the country (Kowalski 2001, Przybył 2002), covering an area of over 10,000 hectares (Gil *et al*. 2006). The first studies to determine the causes of the disease were carried out at the turn of 2000 / 2001. On their basis the fungus of the genus *Chalara* was indicated as a cause, and its morphological characteristics was described (Kowalski 2001). Then, the fungus was proposed as a new anamorphic ascomycetous species, *Chalara fraxinea* T. Kowalski (Kowalski 2006). It was observed that the conidia had no germination ability. It was presumed, therefore, that in the nature must be teleomorph, which plays an important role in the infection process of trees. The ascocarps were observed for the first time in summer 2008 at last year's fallen ash leaf rachises and at the base of the stem of dead young *F. excelsior* seedlings. Cultures derived from ascospores produced in these ascocarps were identical to cultures isolated from lesions on the stems of ash trees, indicating that we found both the anamorph and teleomorph of the fungus that causes ash dieback. On the basis of morphological features teleomorph was initially designated as *Hymenoscyphus albidus* (Kowalski and Holdenrieder 2009a). Further research, based on DNA sequences of isolates from Switzerland, Poland and other European countries have shown, however, that the fungus is different from *H. albidus*; and therefore has been described as a new species, *Hymenoscyphus pseudoalbidus* Queloz *et al*. (Queloz *et al*. 2011). Under the new rules of naming, Baral *et al*. (2014) proposed a new taxonomic designation, *Hymenoscyphus fraxineus* Baral, Queloz & Hosoya.

Pathogenic properties of *H. fraxineus* were confirmed for the first time by artificial inoculation of *F. excelsior* shoots in 2009. The symptoms developed after artificial inoculation were identical to those after natural infections (Kowalski and Holdenrieder 2009 b). *H. fraxineus* also induced necrosis on all inoculated rachises of

F. excelsior with mean lengths of 8.4 cm after two months (Kowalski *et al.* 2015). *Fraxinus pennsylvanica* proved to be more resistant than *F. excelsior*. For all rachises *F. pennsylvanica* artificially inoculated formed necrosis, but their average length was 1.9 cm. Lesions occurred only on about 5% of the inoculated *F. pennsylvanica* stems with mean length 1.9 cm. In comparative studies, necroses developed on all of the inoculated *F. excelsior* stems with mean length 18.0 cm (Kowalski *et al*. 2015). *Hymenoscyphus albidus* did not cause necrotic lesions on the stems of the *F. excelsior* and *F. pennsylvanica*, only small lesions developed on the rachises of both ash species, but with no significant distinction from the controls. It was shown that he did not have pathogenic properties (Kowalski *et al*. 2015).

Research carried out in stands in different parts of Poland showed that the most frequent disease symptoms in ash stands were: the dead top, the dying of whole branches, the dying of the top of branches, the occurrence of healed and unhealed cankers on stems and branches as well as adventitious shoots on trunks. Much less often the slime fluxes on trunks were observed. Most of the types of disease symptoms appeared irrespectively of the tree age, origin and site, sometimes showing only a difference in the frequency of the occurrence (Kowalski and Łukomska 2005, Kowalski, Czekaj 2010, Kowalski *et al*. 2012).

In Poland *H. fraxineus* produces ascocarps mainly from early July to late August and in a lesser intensity also in September, in some years they can still be met in the first half of October. In 2011, the amount of infectious material of *Hymenoscyphus fraxineus* was estimated by the leaf litter examination on 166 square plots in Myślenice and Dynów Forest Districts. The total number of ash leaf rachises and the number of apothecia per rachis were counted. The analysis shows that the estimated number of *H. fraxineus* apothecia may reach from 360 thousand to over 13 million per hectare (Kowalski *et al*. 2013).

The phenomenon of dying stands of ash was observed in Poland since 1993. In order to assess its scale, in 2004, we sent a questionnaire to all forest districts of the State Forests, operating on the surface of approx. 7.6 million hectares equaled 83% of the forest area. At the same time measurements of selected stands of ash across the country, characterized by varying size of dieback were performed. The aim of the study was to assess the health condition of ash stands in the State Forests and to develop silviculture methods for subsequent phases of forest growth in stands with a significant share of ash, in order to minimize the negative consequences of ash dieback and to increase the durability and sustainability of these stands.

Methodological assumptions

The questions related to survey dealt with:

- the area of stands with ash dieback
- the starting year of ash dieback and culmination period of the disease
- developmental stages in which this disease is observed
- the starting point of dying (edge stand, interior, etc.)
- the development phase stand where the earliest phenomenon occurred, and
- type of forest site, in which the dieback is observed
- forms of ash mixing in the stand, in which the phenomenon occurs

One important element of our work was to establish experimental plots in the stands affected (medium and severe degree) or not affected at all by the phenomenon of dieback. These areas were located in forest divisions: Rudka (RDSF Bialystok, north - eastern Poland), Karczma Borowa (RDSF Poznań, western part Polish) and Miękinia (RDSF Wroclaw, south-western part of the country).

In order to determine the selection criteria we developed the scale for assessing the degree of tree dieback, based on the condition of the assimilation apparatus:

- 0 - foliage no damage or minimal damage (defoliation below 10%),
- 1 - defoliation 10 - 39%,
- 2 - defoliation 40 - 69%,
- 3 - defoliation above 70%.

In every investigated above forest districts, the four plots were selected in stands with dominant or significant share of ash. The plots were located on the most representative habitats for each type of stand, namely: fresh forest, moist forest and alder-ash stand. From 60 to 100 randomly selected trees on experimental plots (compartments) were measured, depending on the possibilities. The measurements were carried out during the growing season of 2005, so in order to properly assess the current state of the foliage of trees. Measurements included:

- diameter at breast height of trees,
- the height of trees,
- the length of the living crown,
- vertical projections of crowns (in eight directions).

During the measurements of the crown we sought to capture its size before dying shoots.

Results

390 forest districts (FDs), 90.5% of all units granted responses to the questionnaire. Assuming that in the group of other 41 FDs were also those in which there were no stands with the share of ash (hence did not return the survey) the data obtained allow us to generalize the results across the National Forests in the country.

The ash dieback was found in 86% forest districts covering the area nearly 11 thousand ha. In most FDs disease began in the years 1998-2001, and its culmination took place mostly in 1999-2003. Most vulnerable stands (about 4 thousand ha) were afforested (artificial origin) and young up to 20 years old (37% of the total area of all dying stands of ash). The share of young dying stands of ash (at the age of almost 20 years) generally exceeded 30% in Poland but, in the central part of the country's reached the highest values, over 60% (Fig. 1a). Slightly better state of health showed ash stands being in average age (Fig. 1b). In the central and northern parts of the country we mainly faced ash dieback in stands aged 21-50 years, which accounted for over 50% of the total area of forest dominating by this species. The total area of dying stands in this age group was 1.9 thousand ha. Ash dieback in the stands aged 51-80 years was found on the area of 2.8 thousand ha, mostly in the eastern and central Poland. Clearly, the smallest share of the area of dying stands in this range of age was noticed in the southern part of the country (Fig. 1c). The surface of dying older ash stands (over 80 years old) was estimated as 2 thousand ha on a national scale. By far the most damaged of old ash stands were observed in the eastern and central Poland as well as in eastern and middle Pomerania (Fig.1d). The smallest share of dying stands occurred in the southern and western parts of the country.

This may be due to more favorable for hosts plexus of weather conditions present in southern part of the country, at the same time being less favorable for the development of pathogens. Important role in this regards could also play a greater share of naturally regenerated stands (self-seeding), which could acquire higher resistance (Nörr 2000). The analysis of the surveys showed that ash dieback occurred relatively less frequently in stands with a singly share of this species.

Detailed measurements carried out on research plots located in three forest divisions: Mękinia (RDSF Wroclaw), Karczma Borowa (RDSF Poznań) and Rudka (RDSF Białystok) provided more information on the relationship between the disease progression and the management of ash stands.

The best state of ash health was found in Mękinia FD, the situation in other two stands differed. All three investigated stands grew on habitats and soils (forest type) being appropriate for the ash cultivation. Mękinia FD was located further away from agricultural land, which according to some authors (Savill 1992) may affect susceptibility to ash dieback.

Biometric features of trees took higher values in healthier looking trees (Fig. 2), this regularity was the most clear in the case of crown architecture and the least marked in the case of the height of trees. The crowns of trees accounted from over 30% of the tree high to more than 65%, depending on the habitat, wherein the shorter the crown was the worse health status of tree was noted. The feature that seems to have the highest relationship with the state of health of ash trees is the surface projection of the crown, which in healthy trees fluctuated around the 15 - 17 m^2 in Mękinia FD (should be considered as minimum). In Karczma Borowa FD crown surface ranged from 7.6 m^2 in dying trees up to 44 m2 in healthy trees, and in Rudka FD- from 8.4 to 35.3 m^2.

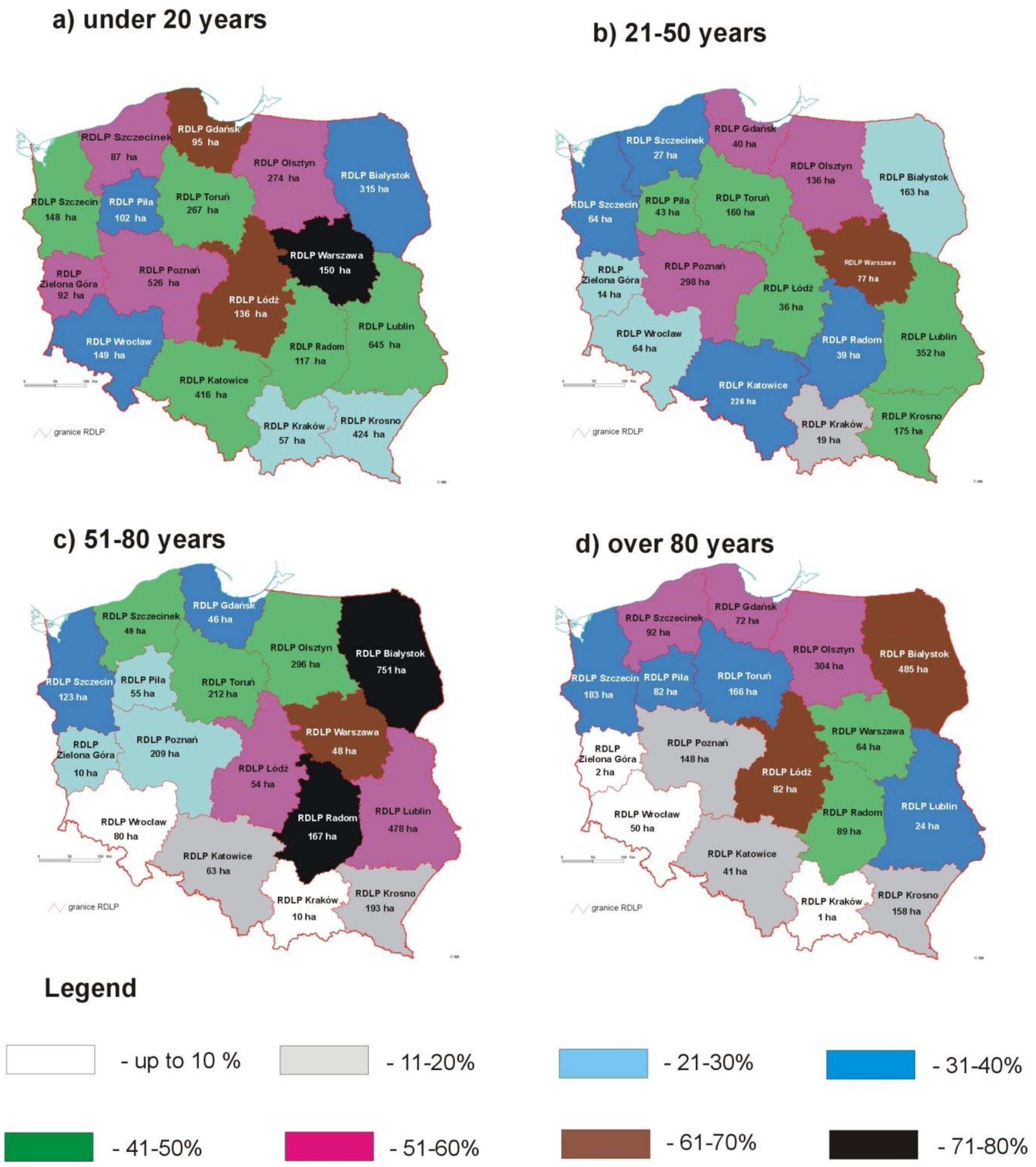


Figure 1 The percentage share of dying ash stands within age intervals by Regional Directorates of State Forests.

According to the research of Vyskota (1976) the surface of crowns of dominant ash trees (at the age of 90 growing in the floodplain forest habitat) is 1.5 times higher than the corresponding values for codominant ash trees and four times greater than that of suppressed ash trees. In the light of these results, the living space of tree is an important feature, which conditioning the resistance of trees to adverse abiotic factors. The study allowed to formulate silviculture guidelines in stands with the share of ash trees being threatened by dieback phenomenon.

Because the ash is a tree species, which loses the ability to regenerate the crown quite early - at the age of about 60 years, therefore the radial growth must stimulated in the first half of its cutting rotation Rebu (Vyskot 1976, Nüsslein 1993). In the second half of cutting interval the use of strong cuts does not bring an incremental effect on neglected ash trees having short and narrow crowns. The early and frequent (systematic) tendering operations prevent development of harmful density in the stand, which in addition to abnormal development of the canopy may also result in the deformation of the root system (Rust and Savill 2000, Roloff 2001), and in consequence weakening and increasing susceptibility to the disease.

Many authors (Vyskot in 1976, Mayer 1992, Savill 1992, Nüsslein 1993, Rittershofer 2001 *et al.*) recommend the completion of silvicultural operations supporting the development of crowns in the first half cutting rotation, starting from the stage of young forest (thicket). Of particular importance is supporting ash trees against associated faster-growing species. With early promotion of ash growth the incremental losses are small and recompensed by higher values of wood timber. Treatments in the early stage of thinning do not have to be strong but they should have a high frequency. It is appropriate to promote uniform distribution and avoid creating groupings of elite ash trees.

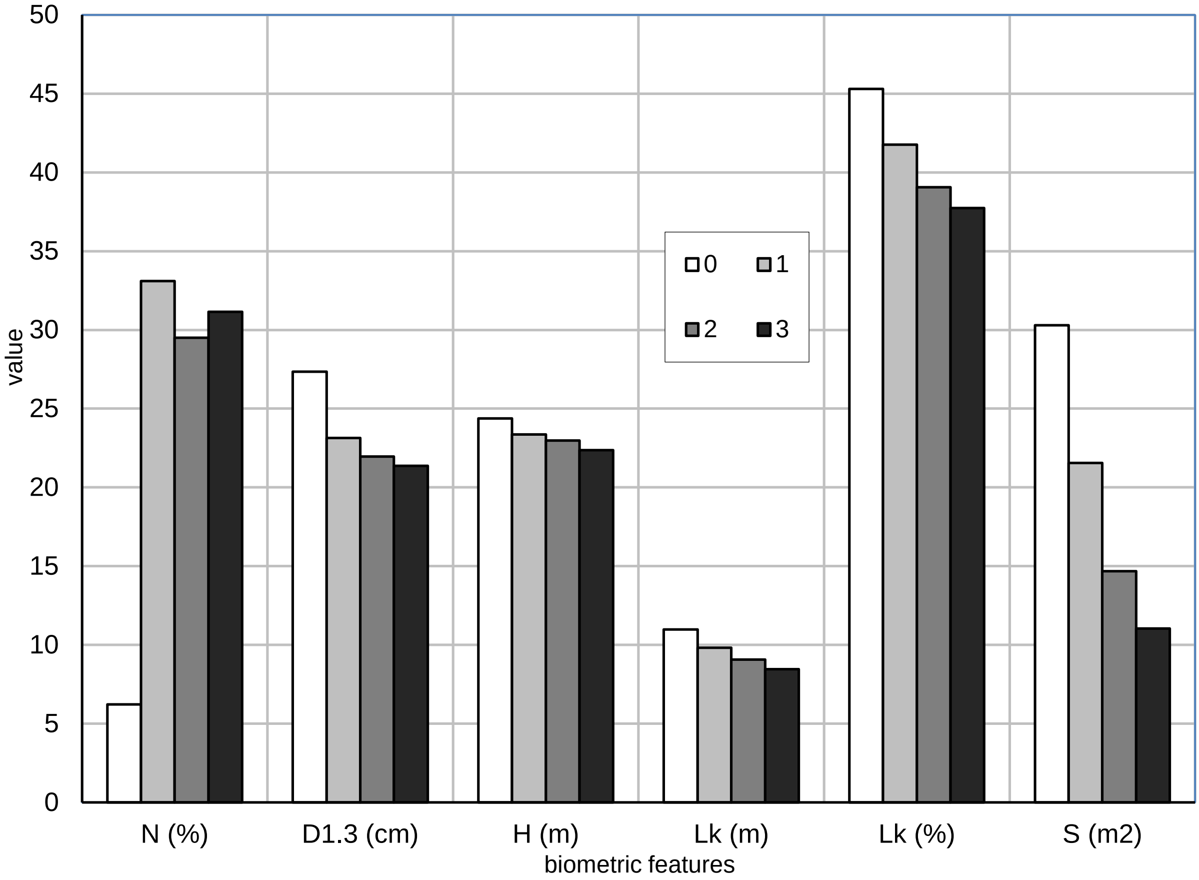


Figure 2 Karczma Borowa Forest District. Biometric features of trees with various levels of defoliation [N – percent of trees with various stage in dieback, D1.3 – DBH, H – height, Lk (m) – length of the crown, Lk(%) – share of the crown in height of the tree, S – area of the crown projection. 0, 1, 2, 3 – levels of defoliation]

At the age of 60-70 years the length of "purified" trunk ash should reach 10-12 m. Further reducing of the crown is not desirable, because it will little increase the value of the timber, but significantly reduce the radial increment in the second half of the cutting rotation. The cuts should be conducted with moderate and low intensity (Nüsslein 1993).

Establishment of single-species stands of ash (monoculture) over large areas should be avoided in order to reduce the risk of dieback and following the strategy of forest durability and sustainability. It is recommended group and clumps mixing of this species and the use of natural regeneration, in the case of documented origin of stands. Also, it is not recommended to afforest former farmland with a significant share of ash (over 30%), especially in the case of the prior impossible to monitor and control agricultural water management (meliorations technology).

Dieback of ash in Poland is still a current issue. In connection with large losses in the units subordinated to the State Forests, there is ban of ash breeding in nurseries and its introduction into the forest plantations until development of effective methods reducing the progress of tree dying.

Genetic variability of *Hymenoscyphus fraxineus* in Poland

Studies on the genetic diversity of the population *H. fraxineus* using RAMS markers (Random Amplified Microsatellites) were performed in Polish regions differing in height above sea level and climatic conditions (Kraj *et al*. 2012, Kraj, Kowalski 2014). Fungal isolates were obtained from lesions occurring on ash shoots and ascospor germinated on medium after being stranded from ascocarps of the fungus. As a result, the impact of climatic conditions on the genetic variation of the fungus, mutual genetic relationships between sexual and asexual generation and stability of genetic diversity in subsequent generations of the pathogen. The high level of genetic variation *H. fraxineus* was shown (Kraj *et al*. 2012; Kraj, Kowalski 2014). None of the resulting amplification products was not monomorphic for groups of isolates originating from different regions. Similarly, for any of the applied primer pairs, there were no isolates representing the same haplotype. Mantel test ruled out the existence of a relationship between geographic and genetic distance between populations. The main factor influencing the level of genetic differentiation of isolates were climatic conditions. The degree of genetic diversity was growing parallel with the height above sea level. This was associated with sharper climate conditions and increased of its variability in the areas on higher altitudes. Based on Dice's similarity coefficient, Nei's diversity and Shannon's Information Index, all analyzed specimens of *H. fraxineus* were divided into lowland, upland and mountain (Kraj *et al*. 2012). It should be assumed that, the high level of genetic variability of isolates (which is characteristic for the higher altitudes) is associated with the evolutionary strategy of the genus, which allows population of the pathogen to maintain sufficiently high plasticity and adaptability. For fungus it allows tolerate better variable climatic conditions in these regions. The effect of temperature on the growth rate of cultures of the pathogen was also demonstrated by Kowalski and Bartnik (2010). Colonies of *H. fraxineus* grew in vitro at temperature of 5-25^{o}C. Isolates sporadically still grew, though slowly, at 30°C. The optimum temperature for fungus growth was 20°C, but some of tested isolates grew quickly at 25°C or at 15°C. The temperature was the main factor affecting morphology of colonies *in vitro* (Kowalski, Bartnik 2010). Differences in the growth occurred not only between isolates from the remote forest districts, but also within the same forest district. Intra-population genetic variation of isolates obtained from ascospores was higher than the genetic variability of strains coming from the same populations and regions, but received from necrosis of plant tissues (Kraj *et al*. 2012; Kraj, Kowalski 2014). Individuals representing anamorphic and teleomorphic forms of the pathogen came from Polish regions with similar climatic characteristics and therefore were subjected to similar selective pressure from climatic factors. As a result, it was possible to compare genetic parameters of studied populations of the fungus. The impact on genetic variation of individuals originating from ascospores could have several factors like: the variability of individuals of the fungus forming ascospores, (depending on geographical location and height above the sea level), climatic areas of origin of fungi strains and the degree of host resistance (individual ash trees infected by the pathogen). Individual ash trees exhibiting greater resistance to infection have the ability to inhibit (or even prevent) spore germination on leaves, and then its mycelial growth, which lowers the genetic variability of the population of the fungus. More resistant individuals have an active defence mechanism of inhibiting growth of the pathogen (McKinney *et al*. 2012). The viridiol production by the fungus (toxic to ash cells) is also important for the level of its genetic variation (Grad *et al*. 2009, Andersson *et al*. 2010). During the growth of *H. fraxineus* a culture medium *in vitro* it was stated that on mycelium and around colonies of some strains the agglomerations of whitish crystal-line substance were being formed. It was demonstrated that *H. fraxineus* can produce viridin and its dihydroderivative viridiol (Mukherjee *et al*. 2006). Shoots of *F. excelsior* seedlings on which the influence of viridiol with the usage of lanolin were tested, displayed necrotic changes of tissues after 10 days. This phenomenon points out the phytotoxic properties of viridiol in relation to ash *F. excelsior* tissues (Grad *et al*. 2009).

Genetic characterization of Polish ash populations revealed by the DNA marker studies

The knowledge of amount and distribution of genetic diversity is essential for any species study in order to better understand its history and adaptive potential to the environmental conditions. Many studies have already attempted to describe the gene pool of Fraxinus sp in Europe. The broadest range of European ash populations were investigated by Heuertz (2003) and Heuertz *et al*. (2004) with nuclear microsatellite loci and chloroplast gene. These studies resulted in two different genetic pattern observed between Western and Southwestern populations in Europe, whilst Polish and Lithuanian ash genotypes belonged to the same pattern as English, Irish, French, German, Swiss, Hungarian and Slovakian ash trees.

On the one hand, the present status of forest tree populations in Europe result from post-glacial migration routes which took place c.a. 18000 BC from Southern European and Boreal refugia (Petit *et al*. 1997, Sperisen *et al*. 2001). On the other hand, the observed differences between gene pool variability among countries may be explained by selection forces or genetic drift or anthropological pressure followed by fragmentation of the ash populations across Europe. The spread of harmful *H. fraxineus* (*Chalara fraxinea* anamorph) among ash stands in many European countries severely reduced the genetic richness of *F. excelsior* populations.

Since 2004 more and more Polish ash stands have been investigated with molecular DNA markers as a tool. First, the 153 European ash trees from 5 different stands examined with random amplified polymorphic DNA (RAPD) markers revealed 97.0% polymorphic loci with mean observed (n_a = 1.970) and expected (n_e = 1.301) alleles number per locus (Nowakowska *et al*. 2004). The same study described the gene pool of 30 Lithuanian ash trees from 3 distinct stands as 95.2% polymorphic loci with n_a = 1.952 and n_e = 1.337. Polish *F. excelsior* populations were slightly more variable (G_{ST} = 0.198) in comparison with Lithuanian stands (G_{ST} = 0.122), and were grouped in two separate clusters based on genetic distance similarity level. The RAPD markers cover large part of genome without being restricted to the coding regions but its usefulness was reported for studying of the genetic population structure of many forest tree species populations (Jeandroz *et al*. 1996, Ratkiewicz and Borkowska 2002, Zvingila *et al*. 2002). The interaction between European ash genotype and environment conditions was assessed with the RAPD markers in Lithuanian population trial and resulted on high genetic changes within population variation, which influence affect adaptive potential and the competitive ability of the studied trees (Pliura and Baliuckas 2007). Generally, high level of genetic differentiation level is considered as a guaranty for better adaptation and tolerance mechanisms in changing environmental conditions.

Later on, the microsatellite markers (both nuclear and chloroplast SSRs) have been considered to be most suitable tool to evaluate genetic differentiation level within and among F. excelsior stands. Our preliminary studies carried out in two Polish sites: natural reserve of Wolica and Primeval Forest in Białowieża, revealed high heterozygosity level among investigated ash trees. Interestingly, trees form Wolica Reserve (Central Poland) had statistically significant differences in observed heterozygosity assessed with nuclear SSR markers, and statistically significant differences in chloroplast SSR loci between trees grouped into three different classes of vitality (Pacia *et al*. 2006, in press).

As far as the tolerance process in plants is generally based on regulated gene-expression mechanism and benefic DNA mutations, we will focus on precise DNA marker development (e.g. SNPs) suitable for identification of tolerant ash tree phenotype in opposition to the susceptible one, as demonstrated by Harper *et al*. (2015). The selection of the resistant F. excelsior genotypes, their genetic testing at the progeny stage, and finally breeding of tolerant ash trees in forest nurseries will overcome the gap caused by high mortality of ash trees due to dieback phenomenon.

At the same time, the development of fast and reliable molecular tool (like TaqMan probes) will facilitate to detect *H. fraxineus* presence in asymptomatic ash tissue and therefore help to prevent the disease spread from nurseries to the forest.

References

Andersson, P.F., Johansson, S.B.K., Stenlid, J. and Broberg, A. 2010. Isolation, identification and necrotic activity of viridiol from *Chalara fraxinea*, the fungus responsible for dieback of ash. *Forest Pathology* 40: 43-46.

Baral, H.O., Queloz, V. and Hosoya, T. 2014. *Hymenoscyphus fraxineus*, the correct scientific name for the fungus causing ash dieback in Europe. *IMA Fungus* 5 (1): 79–80.

Gil, W., Łukaszewicz, J., Paluch, R. and Zachara, T. 2006. Zamieranie jesionu – rozmiar problemu [Ash dieback – dimension of the problem]. *Las Polski* 5: 19 (in Polish).

Gil, W., Łukaszewicz, J., Paluch, R. and Zachara, T. 2006. An analysis of regional and temporal aspects of ash dieback in Poland. In: Possible limitation of decline phenomena in broadleaved stands. Ed. Oszako T., Woodward S. Forest Research Institute, Warsaw, pp. 91 – 96.

Grad, B., Kowalski, T. and Kraj, W. 2009. Studies on secondary metabolite produced by *Chalara fraxinea* and its phytotoxic influence on *Fraxinus excelsior. Phytopathologia* 54: 61-69.

Grzywacz, A. 1995. Ważniejsze choroby infekcyjne [The most important infection diseases]. W: Jesion wyniosły *Fraxinus excelsior* L. (red. Bugała W.). Wyd. Sorus, Poznań-Kórnik: 371–415 (in Polish).

Harper, A.L., McKinney, L.V., Nielsen, L.R., Havlickova, L., Yi, Li, Trick, M., Fraser F., Wang, L., Fellgett, A., Sollars, E.S.A., Janacek, S.H., Downie, J.A., Buggs, R.J.A., Dahl Kjær, E. and Bancroft, I. 2015. Molecular markers for tolerance of European ash (*Fraxinus excelsior*) to dieback disease identified using Associative Transcriptomics. *Scientific Reports* 6: 19335.

Heuertz, M. 2003. Population genetic structure in common ash: a focus on southeastern European genetic resources. Free University of Brussels, Public Research Centre – Gabriel Lippmann, Brussels.

Heuertz, M. Fineschi, S., Anzidei, M., Pastorelli, R., Salvini, D., Paule, L., Frascaria-Lacoste, N., Hardy, O.J., Vekemans, X. and Vendramin, G.G. 2004. Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe. *Molecular Ecology* 13(11): 3437-52.

Jeandroz, S., Frascaria-Lacoste, N. and Bousquet, J. 1996. Molecular recognition of the closely related *Fraxinus excelsior* and *F. oxyphylla* (Oleaceae) by RAPD markers. *Forest Genetics* 3 (4): 237–242.

Kowalski, T. 2001. O zamieraniu jesionów [About ash dieback]. *Trybuna Leśnika* 4 (359): 6-7 (in Polish).

Kowalski, T. 2006. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland. *Forest Pathology* 36: 264-270.

Kowalski, T. and Bartnik, C. 2010. Morphological variation in colonies of *Chalara fraxinea* isolated from ash (*Fraxinus excelsior* L.) stem with symptoms of dieback and effects of temperature on colony growth and structure. *Acta Agrobotanica* 63 (1): 99-106.

Kowalski, T., Białobrzeski, M. and Ostafińska, A. 2013. The occurrence of *Hymenoscyphus pseudoalbidus* apothecia in the leaf litter of *Fraxinus excelsior* stands with ash dieback symptoms in southern Poland. *Acta Mycologica* 48 (2): 135-146.

Kowalski, T., Bilański, P. and Holdenrieder, O. 2015.Virulence of *Hymenoscyphus albidus* and *H. fraxineus* on *Fraxinus excelsior* and *F. pennsylvanica.* PLoS ONE 10 (10): e0141592. doi:10.1371/journal.pone.0141592.

Kowalski, T. and Czekaj, A. 2010. Symptomy chorobowe i grzyby na zamierających jesionach (*Fraxinus excelsior* L.) w drzewostanach Nadleśnictwa Staszów [Disease symptoms and fungi on dying ash trees]. *Leśne Prace Badawcze* 71 (4): 357-368 (in Polish).

Kowalski, T. and Holdenrieder, O. 2009a. The teleomorph of *Chalara fraxinea*. *Forest Pathology* 39: 304–308.

Kowalski, T. and Holdenrieder, O. 2009b. Pathogenicity of *Chalara fraxinea*. *Forest Pathology* 39 (1): 1-7.

Kowalski, T., Kraj, W. and Bednarz, B. 2016. Fungi on stems and twigs in initial and advanced stages of dieback of European ash (*Fraxinus excelsior*) in Poland. *European Journal of Forest Research* (in press).

Kowalski, T., Kraj, W. and Szeszycki, T. 2012. Badania nad zamieraniem jesionu w drzewostanach Nadleśnictwa Rokita [Research on ash decline phenomenon in stands of Rokita FD]. *Acta Agraria et Silvestria., Ser. Silv.* 50: 3-22 (in Polish).

Kowalski, T. and Łukomska, A. 2005. Badania nad zamieraniem jesionu (*Fraxinus excelsior* L.) w drzewostanach Nadleśnictwa Włoszczowa [Research on ash decline phenomenon in stands of Włoszczowa FD]. *Acta Agrobotanica* 59 (2): 429-440 (in Polish).

Kraj, W. and Kowalski, T. 2014. Genetic variability of *Hymenoscyphus pseudoalbidus* dieback cause of European ash (*Fraxinus excelsior* L.). *Journal of Phytopathology* 162: 218-227.

Kraj, W., Kowalski, T. and Zarek, M. 2013. Structure and genetic variation of *Diplodia mutila* on declining ashes (*Fraxinus excelsior*) in Poland. *Journal of Plant Pathology* 95 (3): 499-507.

Kraj, W., Zarek, M. and Kowalski, T. 2012. Genetic variability of *Chalara fraxinea*, dieback cause of European ash (*Fraxinus excelsior* L.). *Mycological Progress* 11(1): 37-45.

Mayer, H. 1992. Waldbau auf soziologisch – ökologischer Grundlage [Silviculture on sociological and ecological basis]. 4 neu bearbeitete Auflage. Gustav Fischer, Stuttgart, Jena, New York (in German).

Mukherjee, M., Horwitz, B.A., Sherkhane, P.D., Hadar, R. and Mukherjee, P.K. 2006. A secondary metabolite biosynthesis cluster in *Trichoderma virens*: evidence from analysis of genes under expressed in a mutant defective in morphogenesis and antibiotic production. *Current Genetics* 50: 193–202.

Nörr, R. 2000. Stabilität beginnt bei der Wurzel [Stability starts at the root]. *AFZ Der Wald* 8: 407-409 (in German).

Nowakowska, J., Jabłonowski, S., Mockeliunaite, R. and Bieniek, J. 2004. Genetic variability within and among Polish and Lithuanian populations of common Ash (*Fraxinus excelsior* L.) based on RAPD analysis. *Baltic Forestry* 10(1): 57-64.

Nüsslein, S. 1993. Struktur und Wachstumsdynamik jünger Büchen-Edellaubholz-Mischbestände in Nordbayern [Structure and growth momentum of young beech, precious hardwood mixed stands in northern Bavaria]. Schriftenreihke der Forstwissenschaftlichen Fakultät der Universität München (in German).

Pacia, A., Nowakowska, J.A., Tkaczyk, M., Sikora, K., Tereba, A., Borys, M., Milenković, I., Pszczółkowska, A., Okorski, A. and Oszako, T. 2006. Common Ash Stand Affected by Ash Dieback in the Wolica Nature Reserve in Poland. *Baltic Forestry* (in press).

Petit, R.J., Pineau, E., Demesure, B., Bacilieri, R., Ducousso, A. and Kremer, A. 1997. Chloroplast DNA footprints of postglacial recolonization by oaks. *Proc. Nat. Acad. Sci. USA* 94: 9996–10001.

Pliura, A. and Balickas, V. 2007. Genetic variation in adaptive traits of progenies of Lithuanian and western European populations of *Fraxinus excelsior* L. *Baltic Forestry* 13(1): 28-38.

Przybył, K. 2002. Fungi associated with necrotic apical parts of *Fraxinus excelsior* shoots. *Forest Pathology* 32: 387–394.

Queloz, V., Grünig, C.R., Berndt, R., Kowalski, T., Sieber, T.N. and Holdenrieder, O. 2011. Cryptic speciation in *Hymenoscyphus albidus*. *Forest Pathology* 41(2): 133-142.

Ratkiewicz, M. and Borkowska, A. 2002. Zastosowanie technik molekularnych w ekologii [Application of molecular techniques in ecology]. *Wiadomości ekologiczne* 2: 99-121 (in Polish).

Rittershofer, B. 2001. Die Esche, ein vielseitiger Weltenbaum [The ash, a worldwide tree]. *AFZ, Der Wald* 24 (in German).

Roloff, A. 2001. Biologie und Ökologie der Esche [Biology and ecology of ash]. *AFZ, Der Wald* 13: 660-661 (in German).

Rust, S. and Savill, P.S. 2000. The root system of *Fraxinus excelsior* und *Fagus sylvatica* and competitive relationships. *Forestry* 73(5): 499 – 508.

Savill, P.S. 1992. The silviculture of trees used in British forestry. CAB International, Oxford, 69 pp.

Sperisen, C., Büchler, U., Gugerli, F., Mátyás, G., Geburek, T. and Vendramin, G.G. 2001. Tandem repeats in plant mitochondrial genomes: application to the analysis of population differentiation in the conifer Norway spruce. *Molecular Ecology* 10(1): 257–263.

Stocki, J. 2001. Przyczyny zamierania drzew i drzewostanów jesionowych w Polsce [Causes of ash trees and stand decline]. *Głos Lasu* 4: 17–19 (in Polish).

Vyskot, M. 1976. Tree story biomass in lowland forests in South Moravia. Rozpravy Československė Akademie Věd, Seš 10.

Žvingila, D., Verbylaite, R., Abraitis, R., Kuusiene, S. and Ozolincius, R. 2002. Assessment of genetic diversity in plus tree clones of *Pinus sylvestris* L. using RAPD markers. *Baltic Forestry* 8 (2): 2-7.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 185 – 194.

Evolution of ash dieback in Romania

D. CHIRA*[1], F. CHIRA[1], I. TĂUT[1], O. POPOVICI[2], I. BLADA[1], N. DONIȚĂ[1], C. BÂNDIU[1], V. GANCZ[1], I. A. BIRIȘ[1], F. POPESCU[1], Ș. TĂNASIE[1], C. DINU[1]

[1] *National Institute of Research and Development in Forestry „Marin Drăcea”. Bul. Eroilor 128, Voluntari, Ilfov, Romania,* [2]*Forest District of Adâncata, RNP – ROMSILVA. Com. Adâncata, Suceava, Romania.*
** corresponding author: chira@rdsbv.ro*

Abstract
Common ash (*Fraxinus excelsior* L.) is the most important ash tree species in Romania. Along with the other native (*F. ornus* L., *F. pallisae* Wilmott, and *F. angustifolia* Vahl.) and North-American ash species (*F. pennsylvanica* Marsh. and *F. americana* L.) it covers about 1% of the national forest area. Until 2010, ash decline (415 ha/yr) and ash defoliation (*Stereonichus faxini* and *Lytta versicatoria*) were the only (minor) problems reported by the National State Forest Administration. Ash dieback has first occurred in 2005-2006 in Romania, when around 10 years old common ash plantations had suddenly collapsed in two small areas from southern and eastern part of the country. Late identification of the *Hymenoscyphus fraxineus* in the same areas, in chronic mature diseased stands certifies the early presence of the Asian fungus. The new disease has spread from north to south, but extreme south and south-eastern regions characterised by steppe and forest-steppe conditions are still free of records. Rivers flowing from North Carpathians (watershed of many important rivers of Poland, Hungary, Ukraine, Romania, Rep. Moldova) could be some most important pathways for the fungus spreading in eastern Romania. A socio-economic impact study of ash dieback has been carried out in two forest counties in NE of Romania in order to figure out the most feasible actions meant to prevent or slow down the dieback phenomenon, taking into account social and economic constraints and opportunities. Tree mortality rate is generally slow and chronic (after more than 3 years), relative rapidly (after 1-2 vegetation seasons occurs if associated with root rot) or seldom suddenly (within 2-3 months if associated with root rot). Important lost in natural ash regenerations have not been yet reported, even frequent leaf blight has been observed. Severely infected stands with high tree mortality have been substituted with poplars, mixed oak forests or even *Fraxinus pennsylvanica* (which proved to be tolerant). Forest evolution prognosis in the new climate change conditions, indicate mesophilous species (including *F. excelsior*) will translate from lower to higher altitudes, being substituted by more xerophyte species or ecotypes (including *F. ornus* and *F. excelsior* ssp. *coriariaefolia*).

Keywords: *Hymenoscyphus fraxineus*, ecology, impact, ash tolerance.

1. Introduction

1.1. Importance of ash

Common ash (*Fraxinus excelsior* L.) is the most important ash tree species in Romania. Along with the other native (*F. ornus* L., *F. pallisae* Wilmott, and *F. angustifolia* Vahl.) and North-American ash species (*F. pennsylvanica* Marsh. and *F. americana* L.) it covers about 1% of the national forest area (58000 ha in 1989 to 80000 ha in 2015 – Fig. 1A-D).

Common ash is found throughout the country, from the plain to the mid-mountainous zone (up to about 1,400 m elevation asl.). As scattered tree, part of upper storey, it is an important component of oak (pedunculate or sessile) - dominated and two-storied stands; sometimes (especially on floodplains under natural flooding regime, where its quick growth potential is expressed the best) it can form pure stands on small areas. Narrowed-leaf ash (*F. angustifolia*) has also a general distribution in lower regions, following the humid forest habitats (commonly on floodplains of the major rivers, rarely in Danube Plain). Flowering ash (*F. ornus*) is competitive only in arid and wormer hilly to low mountain sites, where the forest-steppe influence is higher. The other thermophilic ashes (*F. pallisae* and *F. excelsior* ssp. *coriariaefolia*) are rare, in steppe to forest-steppe zones.

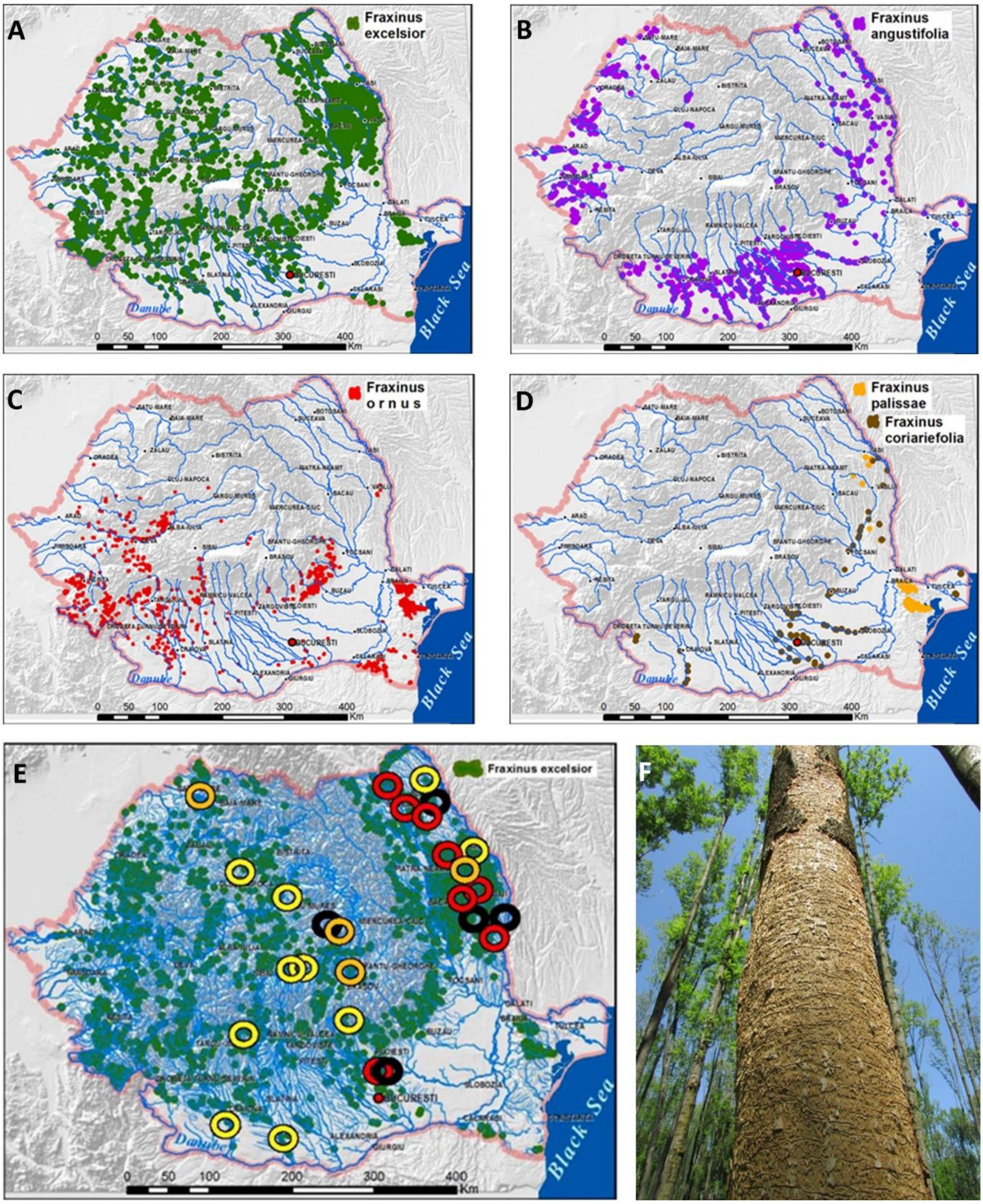


Figure 1 A-D) Distribution of *F. excelsior*, *F. angustifolia*, *F. ornus*, *F. pallisae* and *F. excelsior* ssp. *coriariaefolia* in Romania. Maps have been produced in an IPGRI project (Blada et al. 2008-2010). **E)** Ash dieback evolution (red - early infections: starting in 2005-2010, orange – medium infections: 2011-2012; yellow – later infections: 2013-2015, black – dead/substituted stands). **F)** New reported intensive dieback of common ash in Transylvanian Plateau (July 2015): dead trees with bark beetles (*Hylesinus fraxini*). Note crown reduction, branch lost and stem water-sprouts in the background.

1.2. Ash dieback and its spread (disease history)

In the state forests, common ash decline has been recorded until 1994 (Fig. 2). Due to ash low frequency / participation in stand composition, its problems are not so remarkable in forest statistics, where the phenomenon was recorded only on areas - 415 ha/year between 2001 and 2010, majority (86%) of low intensity. Moderate to high intensity dieback had a peak in 2002 (1,395 ha) when dry climate and insect defoliation were the incriminated factors (Simionescu et al., 2012).

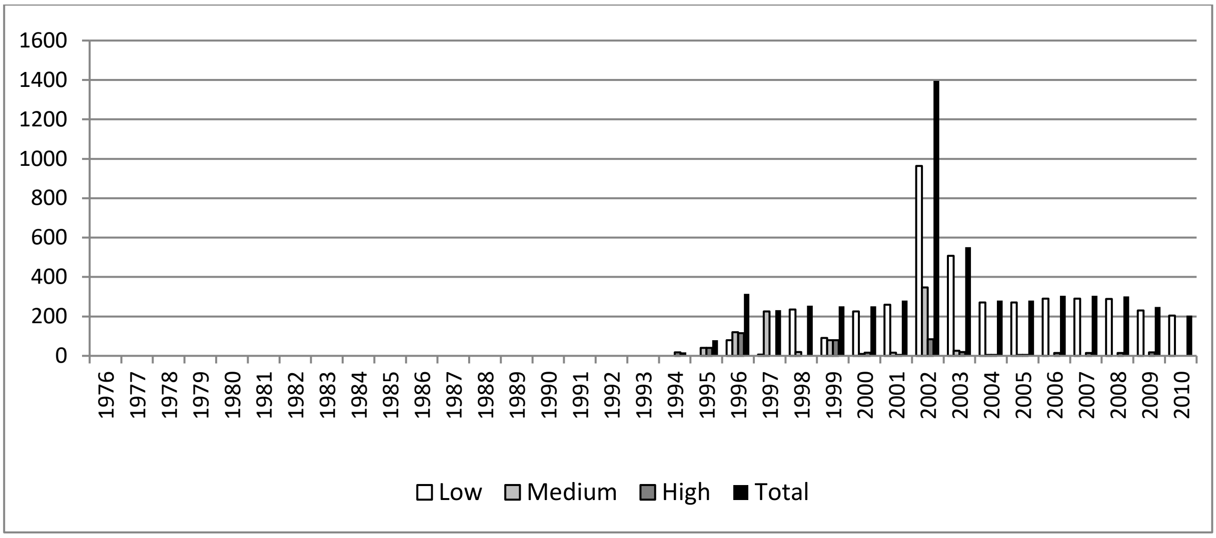


Figure 2 Ash decline area (ha) in Romania (intensity: low, medium, high)

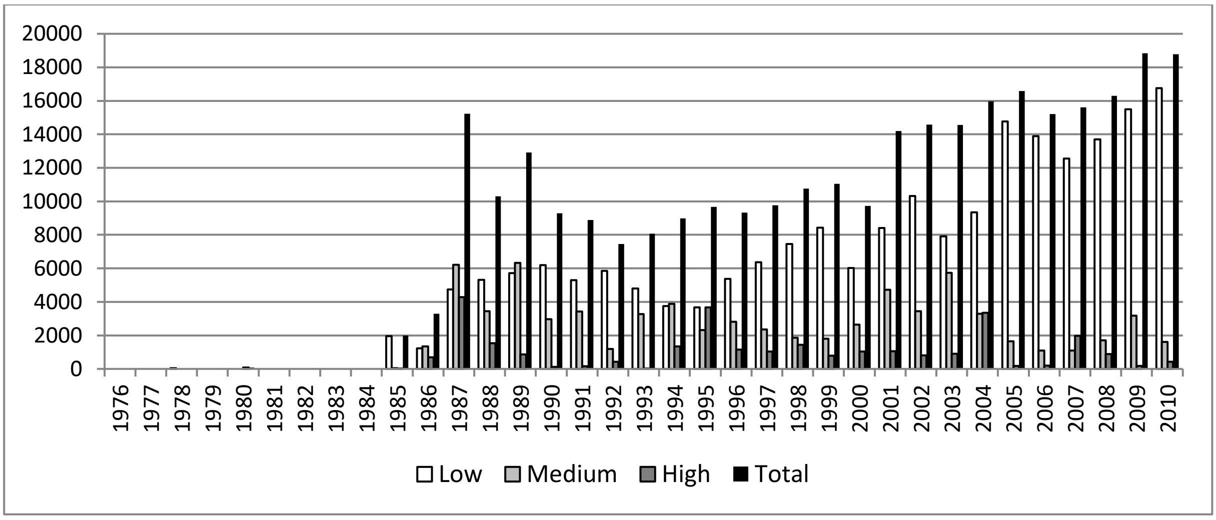


Figure 3 Ash forest area (ha) defoliated by *Stereonichus fraxini* in Romania (intensity: low, medium, high)

Chronic ash decline was noticed in common ash stands of Dolj County Forest Directorate of the State Forest Administration (RNP) ROMSILVA (west of Danube Plain). Dry climate was overlapped to repeat defoliation of *Stereonichus fraxini* both (Fig. 3) in southern plains and eastern hilly zones (Moldavian Plateau) in the last two decades (Neţoiu & Vişoiu, 1996; Blaga, 2013). If *S. fraxini* has affected up to 19000 ha (but rare with medium intensity on 6000 ha), the long-time inventoried defoliator *Lytta versicatoria* has created just minor problems (Fig. 4) (Niţescu et al 1992; Simionescu et al., 2001; Simionescu et al., 2012).

In the second part of the 2000s, after *Chalara fraxinea* Kowalski was proved to create the new ash dieback in Europe, some new ash problems were occurred in Romania. Rapid mass ash dieback was registered in young plantations in Bucharest Plain (part of Danube Plain, in 2005-2006) and Prut Meadows (2006-2007) which was not apparently affected by leaf insects or climate stress. In the same time and areas, some mature ash stands showed severe health degradation that became chronic (and finally they were proved to be infected by *Hymenoscyphus fraxineus*).

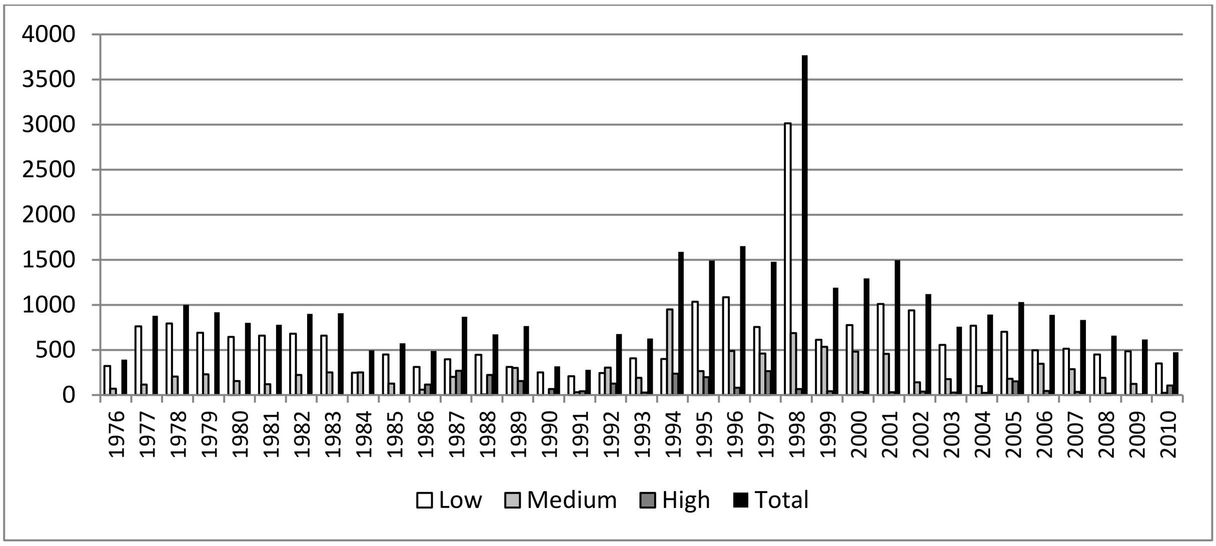


Figure 4 Ash forest area (ha) defoliated by *Lytta versicatoria* in Romania (intensity: low, medium, high)

Ash dieback disease has gradually spread in all important regions – first in large areas of Moldavian Plateau (east Romania, where *H. fraxineus* has been first found - Popovici et al. 2014), then different zones of Transylvania and some spots in northern Walachia.

2. Research regarding ash dieback

2.1. Development and impact of ash dieback

Pathways and disease spread
The original plantations completely damaged by the new disease were situated in two different zones, where the infections have also different evolutions in time. First forest (called „Black pond„ - Snagov Forest District) is located nearby Bucharest (the main important zone in ornamental plant trading), in humid site conditions along Ialomiţa river flooding meadow, so the two essential conditions for arrival and settlement was optimal for the new fungus. A young plantation was dead in 2005, some mature ash monocultures were gradually dyeing and replaced with poplars or oak base mixture, others stands having a continuous slowly crown degradation till now (Chira & Chira, 2007-2008). The disease has chronicised in this small area likely due to its location (forest is very scattered in the Danube Plain) and probably following an accidental arrival - common ash seedlings are never imported for forest plantation, but other ash species are rare traded as ornamentals in Romania. Disease spreading to other forests from the same region was very slow - just in the last 2-3 years new clear symptoms or fruitbodies were found into the forests situated at 30 to 100 km north.

In the same time, the second entrance was related to the Prut and Siret rivers, which spring in Ukraine, on small distance with Poland border, in northern Carpathians, and flow downhill south to reach Danube close to its Delta (Chira & Chira 2007-2008). Almost every year (2006, 2008, 2010, 2013, 2014) those rivers are flooding their forested meadows. First ash diebacks were recorded after severe floods of 2006 (Prut) and 2010 (Siret). On this pathway the fungus may spread rapidly in very large areas – ash problems were recorded in 2007-2013 in whole Moldavian Plateau (large area situated between the two rivers), leaf contamination and *H. fraxineus* fruitbodies were found in every stand with ash problems (inventories took place in very wet summer of 2014). In Ukraine the new disease has also been identified in Carpathians, relative close to Siret and Prut springing zone (Davydenko et al., 2013M; Davydenko & Meshkova, 2014). In Republic of Moldova disease situation should be the same, as the infections occur on more than half of the common Prut river border (Fig. 1E).

The later entrances (2012-2015) all over Transylvania (Fig. 1F) and northern Walachia may be just a sign of natural spread – by wind and/or water - from northern Carpathians and Western Hungary (where *Chalara fraxinea* state was identified by Szabo in 2009), and/or internal material movement respectively.

Pannonian Plain was possible a serious obstacle to *H. fraxineus* spread, common ash being absent (FRAXIGEN 2005) in all this area. Maybe this large discontinuation in natural area of the most sensitive host is the key of late records of ash dieback in lower Danube zone (Serbia, Romania, Bulgaria). In Romanian, ashes are not found on Danube floodplains, just very rare plantations or regenerations of *F. pennsylvanica* or local ash species being lost in a continuous corridor of poplar and willow plantations (Fig. 1A-E).

Sporulation, distribution and climate

Fruitbodies of *Hymenoscyphus fraxineus* have been noticed in summer and autumn (from late June, early July to first half of September) of 2013-2014 (wettest year of the decade) and starting very early in 12 of May 2015 (even in relative cold continental climate of Transylvanian Plateau). That induce the idea the infection period may be rather long and the fungus contamination may be active even in wormer and dry climate of south-eastern part of the European Union.

It was a continuous question if ash dieback will be rather mild or even will stop spreading in Mediterranean climate, due to extreme dry litter and soil in summer time (most important sporulation time). Fungus ability to have fruiting bodies very early in spring, when humidity is rather high even in southern Europe due to Atlantic influence, rise a serious warning for sensitive ash species, especially for narrow-leaved ash which prefers the humid meadows and floodplains. Late autumn sporulation is questionable for fungus biology and spreading.

In Romania, 5 biogeographic regions are found, steppe zone covering south-eastern part (Dobrogea and eastern Danube Plain), respectively forest-steppe being in extension in many regions (Moldavian Plateau, Danube Plain, Pannonian Plain, Danube gorge zone, south-western Transylvanian Plateau). The warmest south part of the country is still apparently free of ash dieback. This may be a consequence of the natural spreading of the fungus (from north to south) or/and because the arid climate is not quite suitable for spore dispersal. In the same time the xerophilous-thermophilous ash species / ecotypes (*F. ornus* and *F. excelsior* ssp. *coriariaefolia*) are more frequent in southern forest-steppe zone, but the sensitive *F. angustifolia* and *F. pallisae* (its sensitivity to *H. fraxineus* is not known) are also relative frequent along the rivers in this zone (Fig. 1A-D and Fig. 2-4). *F. ornus* is proved to be resistant to *H. fraxineus* (Kräutler & Kirisits 2012). The other thermophilous species which are spread in Romania may be subject of future testing, especially for *F. coriariaefolia* (accepted name *Fraxinus excelsior* L. ssp. *coriariaefolia* (Scheele) A.E. Murray), which is considered a different species in Romanian taxonomy according to its morphological (hairy leaves, buds and shoots) and ecological features (too dryer sites for regular common ash demands - Ciocârlan 2000, Doniță et al. 2004, Oprea 2005, Sârbu et al. 2013).

Root rot

At least two types of ash dieback were noticed:

- With high rate (up to 100%) of root rot produced by *Armillaria* sp.;
- Without intensive root rot development or at least even the collar rot (produced by *Ganoderma* sp. or other xylophagous species) is frequent it does not seem to significantly aggravate the tree health.

First was generally met in humid stands with fluvisols, sometimes stagnosols or haplic luvisols, the second being recorded in very different sites.

Disease impact

Generally in complex disease there is an active phase (in the same season or the next one after inciting factors action), followed by a chronic phase, with low debilitation. First Romanian recordings of the new phenomenon were confounded with ash decline, having similar start symptoms (Fig. 5). Chronic evolution of crown degradation and later identification of *H. fraxineus* in these stands proved there was a new ash disease.

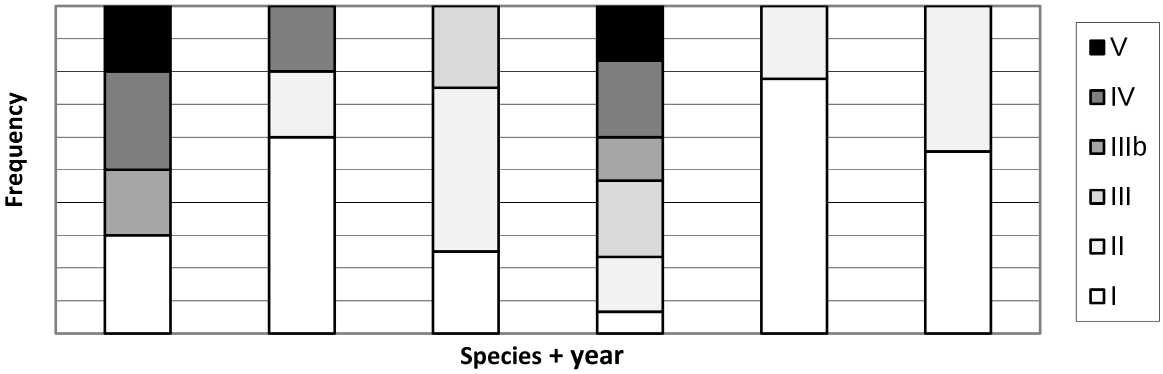


Figure 5 Common ash (FE) dieback comparing to health status of mixed broadleaved from the same stand: common oak (QR) and large-leaf lime (TP), in Snagov F.D., in September 2005 and 2006 (intensity scale: I–healthy to V-dead tree). The columns are in order: 1) FE2005, 2) QR2005, 3) TP2005, 4) FE2006, 5) QR2006, 6) TP2006.

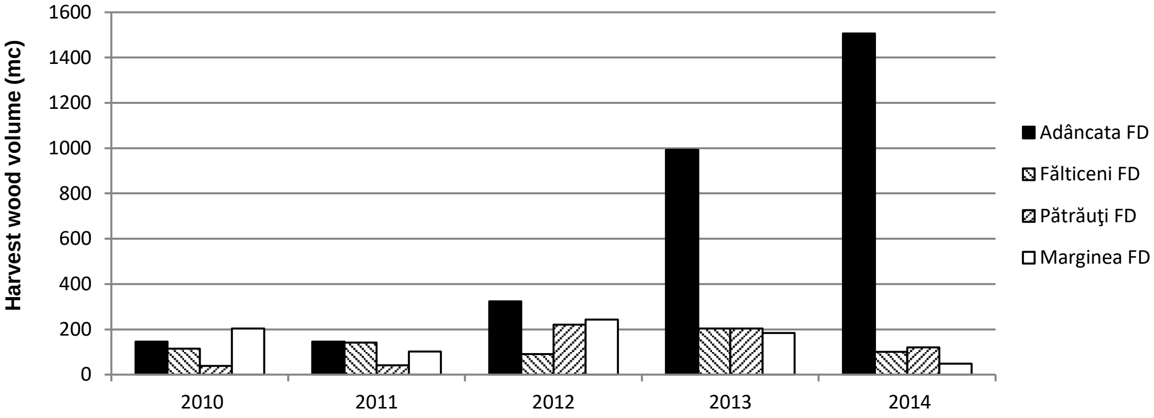


Figure 6 Ash wood volume harvest through sanitary cuttings in four forest districts (Adâncata, Fălticeni, Pătrăuți, and Marginea) of Suceava Plateau, between 2010 and 2014.

Until now, ash dieback had a low rate of evolution, with many trees affected by different defoliation rate / crown reduction and rather discrete death of individual trees. The most concerning symptom of the phenomenon was its continuous health degradation. After many years of chronic disease, some stands dominated by ash have been felled and substituted.

The relationship between collar rot and individual tree rapid death is high in the stands with generalised *Armillaria* root rot. Sudden death is just accidentally recorded on individual trees: a 60 yr old ash had 15% of defoliation in 1st of July and it was completely death in 20th of September 2013 (Fig. 1F). But even in such situation the majority of trees are dying 6-10 years after the first serious symptoms of crown degradation. Annual sanitary cuttings offer interesting data on ash dieback evolution in forest district with larger area of humid floodplains (Adâncata FD) comparing with neighbouring hilly zones (Marginea, Pătrăuţi, Fălticeni F.D. - Fig. 6).

In the same area (NE of Romania), a socio-economic impact study of ash dieback has been carried out, highlighting the actual confusion in understanding the new disease favouring factors, evolution, (economic, social and ecologic) impact, and management (Drăgoi et al. 2016).

2.2. Genetics of ash / populations and resistance

Population structure and genetic diversity of ash in Romania
Romanian populations of *F. excelsior* was dominated by the H01 chloroplast microsatellite haplotype (Fig. 7A), typical to Nordic (Scandinavian, Baltic), eastern (Carpathian, Russian) and south-eastern (Balkans) area. Rare H06 and H11 haplotypes were found only in Carpathian and Balkans' zone (Heuertz et al. 2004). Chloroplast haplotype

H1 was totally dominant in Romanian *F. angustifolia* populations (Fig. 7B), as well as in all Carpathians and Balkans. In just one case the H6 haplotype has been found, typical to Nordic (Scandinavian, Baltic), eastern (Carpathian, Russian) and south-eastern (Balkans) area. Rare H06 haplotype was found only in Carpathian region (FRAXIGEN 2005). Genetic variability of Romanian *F. ornus* (chloroplast haplotypes H19 + H20, allelic richness FEMSA>50% + FR39 + FR41) is (Fig. 7C) similar to the general European genetic distribution of this species (FRAXIGEN 2005).

Genetic resistance in populations and individuals

- No test for genetic resistance was made until now.

Evolutionary selection in natural ash regeneration

Proportion of potentially resistant ash trees

- It is rather early to see natural resistance in the forest.
- Even if the disease may exist for 10 years in Romania its effects have not reached their full potential.

First experiences in breeding for resistance

- The first survey for genetic resistance is planned to start in the spring of 2016 in an ash seed orchard from Siret basin; here the disease is probably naturally spread, since it has been observed in the adjacent zone since July 2013.

Ongoing and planned projects / research in Romania

In 2016 two research projects will analyse the actual status of ash dieback throughout Romania, and evaluate i) the disease spread (pattern?), ii) features (root rot frequency, factors influencing the rapid *vs* chronic dieback), iii) impact (health decrease, mortality rate), iv) genetic resistance (natural resistance of plus tree clones from seed orchard), and v) management options (case studies).

3. Management options to mitigate the impact of ash dieback

3.1. Silvicultural management options

Tending operations and sanitary cuttings are used just to lower down the infection pressure and value the precious ash wood before its degradation. Early felling (generally in 40-60 yr old stands) has been done in old outbreaks (Fig. 1E), several years (starting to the 3rd year) after the first sanitary cutting. Ash dieback has the most severe impact in monocultures, which generally occur in humid river meadows. Romanian norms forbid ash monocultures, but in some cases the main species (common oak) was destroyed due to abiotic, biotic or anthropic factors and the remaining stands were dominated by ash. Regardless, in areas affected by ash dieback, the foresters start to use ash in minor proportions.

3.2. Alternative tree species

Romanian forestry lows strictly regulate forest regeneration and management. Native European species are with rare exceptions promoted; from exotic species only black locust and hybrid black poplars are significant planted, but all exotics occur less than 5% of the national forest area. Promoting local species and provenances in high forest system (coppice is just an exception) Romanian sylviculture is one of the most ecological in Europe. As opposed to the optional European good practice guidelines, Romanians standards are mandatory. The possibility of ash substitution is also indirectly defined in Romanian forestry standards (MAPP 2000), as follows (several examples):

A. Mountain region

A.3. Mountain level of mixed forest of beech and conifers

Ecologic group no. 16: Mountain (sites) of mixed forest on (middle to deep) cambisols (dystric to eutric) (at 1200-1400 m elevation).

Regeneration compositions are:

i) 50-70% *Picea abies* + 10-30% *Abies alba* + 10-30% *Fagus sylvatica*, *Acer pseudoplatanus, Ulmus montana* ± *Fraxinus excelsior*;

ii) 40-50% *P. abies* + 40-50% *F. sylvatica* and *A. pseudoplatanus* + 10% diverse (*U. montana*, *F. excelsior*).

B. Hilly region

B.1(2). Hilly level of pure or mixed oaks (FD2, FD1)

Ecologic group no. 51: Hilly (sites) of sessile oak on (middle to deep) grey soils (250-400 m elevation).
Regeneration composition is:
i) 70-80% *Quercus petraea* + 20-30% *F. excelsior, Prunus avium, Tilia cordata, Acer campestre, Acer tataricum*;
Ecologic group no. 58: Hilly (sites) of Italian oak on vertic argilic (middle to deep) soils (100-350 m elevation).
Regeneration composition is:
i) 70-80% *Quercus frainetto (or Q. petraea)* + 20-30% *T. cordata, P. avium, A. campestre, F. ornus, Carpenus betulus, Pyrus pyraster, Malus sylvatica, A. platanoides*;

C. Plain region
C.1. Forest plain level (C1)
Ecologic group no. 74: Low plain (sites) of common oak on (middle to shallow) pseudogleyc (low-humic gley) soils.
Regeneration compositions are:
i) 50-60% *Quercus robur* + 20-40% *F. excelsior (or Alnus glutinosa), C. betulus, A. campestre* + 10-20% *Quercus rubra*;
ii) 40% *Q. robur* + 20-30% *F. excelsior (or A. glutinosa)* + 20-30% *Tilia cordata, C. betulus, A. campestre* + + 10-30% helping or bush species;

E. Floodplains
Intrazonal formations
Ecologic group no. 102: Danube floodplain or delta (sites) of poplar ± common oak on alluvial (deep) soils.
Regeneration compositions are:
i) 50-70% *Q. robur* or *Q. pedunculiflora* + 30-50% *F. excelsior* (or *F. angustifolia, F. pennsylvanica*), *Populus* sp., *P. nigra*;
ii) 100% *F. excelsior* (or *F. angustifolia, F. pennsylvanica*);
iii) 100% *Populus x canadensis,* (or *P. nigra, Populus* sp.).

Under the current standards, dying ash will be substituted by the local most suitable species to each site characteristics.

Substitution of common ash
In humid areas, severely damaged common ash stands were substituted with poplars, willows, alders (Fig. 7D) or mixed common oak humid forest, but rarely with *F. pennsylvanica* (invasive and very resistant in flooding zone of Danube and lower part of its tributary).

F. pennsylvanica was previously known to show slightly less severe symptoms to *H. fraxineus* inoculations (Drenkhan and Hanso, 2010), but in several cases this species proved to be tolerant to ash dieback in Romania:

- Seedlings of green ash recorded high levels of survival following the substitution of a young common ash plantation severely destroyed by *H. fraxineus* in Prut flooded meadow (Epureni F.D.).
- 26 to 80 years old green ash plantations are in very good health condition in the same forest where common ash is severely infected by *H. fraxineus* in Siret floodplain conditions (Adâncata F.D.).

Climate change influence
Romanian standards (MMAP 2000) shall be modified according to the climate warming, which forecasts that common ash and other mesophilous species will move from lower to higher altitudes and from southern to northern latitudes (Fig. 7E-F).

4. Conclusions

First dieback of young ash plantations and significant crown reduction of mature stands have been recorded in 2005-2006, both in southern and eastern Romania. In the same forest areas *Hymenoscyphus fraxineus* has been later identified. Asian fungus has long period of sporulation, from beginning of May to late September.

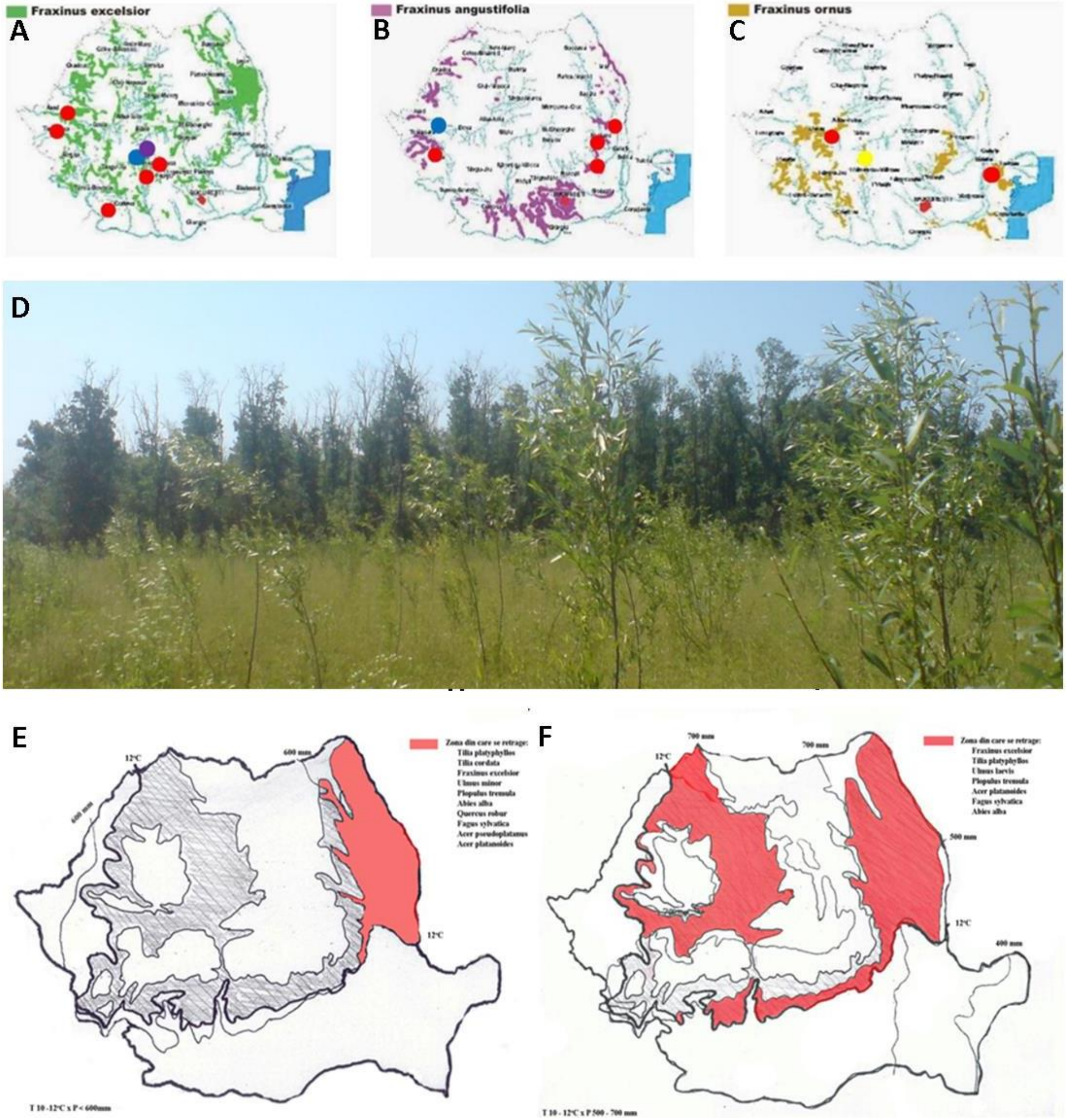


Figure 7 A-C Distribution of ash species / subspecies and their haplotypes in Romania – *F. excelsior* (1 - with H01 red, H06 blue, H11 purple), *F. angustifolia* (2 - with H1 red, H6 blue), *F. ornus* (3 - with H20 red and H19 yellow). Maps were produced in the IPGRI project (Blada et al 2008-2010) and haplotypes in FRAXIGEN project (2005). **D** Substitution of common ash (note the debilitated ash stand in the back) with poplars and willows in Vaslui floodplain (Moldavian Plateau). **E-F** Prognosis of the common ash (and other mesophilous species) extinction (red colour) according to climate change in Romania by the end of XXIst century in two variants of estival temperature (T) and precipitation (P) evolution: E) domain $T_{est}>10\text{-}12^0$ x $P_{est}<600$mm, F) domain $T_{est}>10\text{-}12^0$ x $P_{est}<700$mm (Barbu et al., 2016).

Ash dieback is in continuously developing from northern Carpathians to central hilly and southern field zones of Romania. The dry southern areas (forest field, forest-steppe and steppe zones) are still free of records, likely due to the scarcity of common ash forests and the resistance / low vulnerability of local xerophilous - termophilous ash species (*F. angustifolia, F. pallisae*) and subspecies (*F. excelsior* ssp. *coriariifolia*).

In some forests with severe dieback of *F. excelsior*, *F. pennsylvanica* proved to be tolerant to *H. fraxineus* in all stages (from seedlings to old trees).

Infected stand degradation is chronic, but infected trees with significant *Armillaria* collar rot may die after one season of vegetation or even (accidently) in few months. In the last decade severe outbreaks in common ash monocultures led to ash substitution with more suitable broadleaved species. Disease management is based on sanitation cutting and felling, followed by ash replanting or substitution.

References

Blada, I., Doniţă, N., Bândiu, C., Gancz, V., Biriş, I.A., Tănasie, Ș., Dinu, C. 1998-2000. Conservation of the genetic resources of broadleaved species from Romania. Project report ICAS, IPGRI project (manuscript).

Barbu, I., Curcă, M., Barbu, C., Ichim, V. 2016. [Adapting of Romanian forests to climate change]. Ed. Silvica, 379 pp. [in Romanian].

Blaga, T. 2013. [Technical assistance in control of ash defoliators]. Project report ICAS/RNP Romsilva (manuscript) [in Romanian].

Chira, F., Chira, D. 2007, 2008: [Emergent fungi threatening the forest plants]. Project reports, ICAS/RNP-Romsilva ICAS (manuscript) [in Romanian].

Ciocârlan, V. 2000. [Romanian Flora, Pteridophyta et Spermatophyta]. Ed. 2. Ceres, București, 1138 pp. [in Romanian].

Davydenko, K.V., Vasaitis, R., Stenlid, J., Menkis, A. 2013. Fungi in foliage and shoots of *Fraxinus excelsior* in eastern Ukraine: a first report on *Hymenoscyphus pseudoalbidus*. *Forest Pathology*, 43: 462–467.

Davydenko, K.V., Meshkova, V.L. 2014. European ash (*Fraxinus excelsior*) dieback – situation in Europe and Ukraine. Лісове і садово-паркове господарство, 5.

Drăgoi, M., Chira, D., Popovici, O. 2016. Socio-economic outcomes of ash dieback in Romania. Baltic Forestry, special issue.

Drenkhan, R., Hanso, M. 2010. New host species for *Chalara fraxinea*. *New Disease Reports*, 22: 16. http://www.ndrs.org.uk/article.php?id=22016

Doniță, N., Geambașu, T., Brad, R.R. 2004. [Dendrology]. Vasile Goldiș University Press, Arad, 423 pp. [in Romanian]

FRAXIGEN 2005. Ash species in Europe: biological characteristics and practical guidelines for sustainable use. Oxford Forestry Institute, University of Oxford, UK. 128 pp.

Heuertz, M., Fineschi, S., Anzidei, M., Pastorelli, R., Salvani, D., Paule, L., Frascaria-Lacoste, N., Hardy, O.J., Vekemans, X., Vendramin, G.G. 2004. Chloroplast DNA variation and postglacial recolonization of common ash (Fraxinus excelsior L.) in Europe. *Molecular Ecology* 13: 3437–3452.

Kräutler, K., Kirisits, T., 2012. The ash dieback pathogen *Hymenoscyphus pseudoalbidus* is associated with leaf symptoms on ash species (*Fraxinus* spp.). *Journal of Agricultural Extension and Rural Development*, 4: 261-265.

MAPP 2000. [Technical Standard no. 1 for composition, scheme, and technology of forest regeneration and degraded land afforestation]. Order 1652/31.10.2000 of the Ministry of Water, Forest and Environment Protection [in Romanian].

Neţoiu, C., Vişoiu, D. 1996. [Biology of ash weevil *Stereonychus fraxini*]. *Muzeul Olteniei Craiova*, *Studii și comunicări, Ştiinţele Naturii*: 61–63 [in Romanian].

Niţescu, C., Vlădescu, D., Simionescu, A., Vlăduleasa, A. 1992. [Health status of the forest in Romania in the 1976-1985]. Ed. Inter-Media, Bucureşti [in Romanian].

Oprea, A. 2005. [Chritical list of vascular plants of Romania]. Univ. "Alexandru Ioan Cuza", Iaşi, 668 pp. [in Romanian]

Popovici, G.O., Chira, D., Kirisits, T. 2014. [Ash dieback new agent - *Hymenosyphus fraxineus* - in Suceava Plateau]. *Revista de Silvicultura si Cinegetica* 35: 105-112 [in Romanian].

Simionescu, A., Mihalciuc, V., Lupu, D., Vlăduleasa, A., Badea, O., Fulicea, T. 2001. [Forest health status in Romania in the period 1986-2000]. Ed. Muşatinii, Suceava [in Romanian].

Sârbu, I., Ştefan, N., Oprea, A. 2013. [Vascular plants of Romania. An illustrated field guide]. Publishing House Victor B Victor, Bucharest, 1320 pp. [in Romanian].

Simionescu, A., Chira, D., Mihalciuc, V., Ciornei, C., Tulbure, C. 2012: [Forest health status in Romania in the period 2001-2010]. Ed. Muşatinii, Suceava [in Romanian]

Szabó, I. 2009. First report of *Chalara fraxinea* affecting common ash in Hungary. *Plant Pathology*, 58(4): 797.

Wallander, E., 2008. Systematics of *Fraxinus* (Oleaceae) and evolution of dioecy. *Plant Syst Evol*, 273(1): 25-49.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 195 – 208.

Ash and ash dieback in Sweden: a review of disease history, current status, pathogen and host dynamics, host tolerance and management options in forests and landscapes

M. Cleary[1]*, D. Nguyen[2], L. G. Stener[3], J. Stenlid[2], J. P. Skovsgaard[1]

[1] Southern Swedish Forest Research Center, Swedish University of Agricultural Sciences, Sundsvägen 3, Alnarp, Sweden, [2] Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Almas allé 5, Uppsala, Sweden, [3] Skogforsk, Ekebo, Sweden.
**corresponding author: Michelle.Cleary@slu.se*

Abstract

Common ash, also known as the King's tree in Sweden has suffered largescale decline and mortality as a result of the invasive fungal pathogen *Hymenoscyphus fraxineus*. In 2010, the species was Red-listed and recently its status was elevated to critically endangered. Ash is an important species for several organisms, many of which are already red-listed and further reductions in the host population may have grave implications for ash-associated biodiversity. In Sweden, pollarded ash has large cultural value, though prospects for maintaining vital pollards based on previous inventories and the disease intensification during the last decades, seem grim. Though, some evidence of disease tolerance has been observed in the natural population. Periodic assessments of two clonal seed orchards in southern Sweden have shown strong genotypic variation in susceptibility to the disease, with some individuals showing very little evidence of crown dieback. Since 2013, large efforts have been made towards surveying key habitat areas for ash and seed stands and select putatively resistant trees for future propagation and breeding. Forest management options to alleviate the impact of ash dieback, considering both pre- and post-dieback ash silviculture, are discussed.

Introduction

A new invasive fungal disease is causing widespread dieback and decline of common ash (*Fraxinus excelsior* L.) trees throughout most European countries. Beginning in the early to mid-1990s, large-scale decline of common ash was reported from eastern Poland and Lithuania (Juodvalkis and Vasiliauskas 2002, Przybył 2002). The causal agent of ash dieback was first identified by Polish researchers as *Chalara fraxinea* (Kowalski 2006, Kowalski and Holdenrieder 2009a, Bakys et al. 2009a,b), which is the anamorph of the newly identified ascomycete fungus *Hymenoscyphus fraxineus* Baral et al. (syn. *Hymenoscyphus pseudoalbidus*) (Baral and Bemmann 2014; Queloz et al. 2010). The putative origin of the pathogen is from East Asia where it has co-evolved with, and is a benign associate of, its native *Fraxinus* species (*F. mandshurica*) (Cleary et al. 2016; Baral and Bemmann 2014; Gross et al. 2014; Zheng and Zhuang 2014; Zhao et al. 2013). As with most introduced pests and pathogens where the pathosystem lacks a history of co-evolution, the damage to the host has been devastating. Starting from several infection points in the leaves or young shoots, the fungus destroys the infected tree’s phloem and xylem by disrupting nutrient and water transport, ultimately leading to tree death. The very existence of ash in Europe is now under threat as large populations of ash are disappearing from forest ecosystems and urban landscapes, mostly because of ash trees succumbing to the disease, and partially due to over-cutting.

Leaves and rachises are the primary habitat of *H. fraxineus*, but the fungus also invades buds, bark and wood of shoots and stems. The fungus undergoes an extensive saprotrophic stage, overwintering on rachises in the leaf litter, after formation of a protective pseudosclerotial sheath. Spores are dispersed usually between June and September the following year which are capable of initiating new infections on leaves. Disease symptoms range from necrotic leaf spots, branch cankers, wood discoloration, wilting of shoots, and dieback of twigs, branches and stems (Figure 1A). When branches die back, the tree often sprouts epicormic shoots near the site of infection to replace those that have been lost, often giving a bushy appearance to the tree. These too may eventually succumb to the disease. In Sweden, ash is an important noble broadleaved tree species that has a large ecological relevance. The consequences of this new invasive disease and the lack of knowledge on management options for conserving ash in Swedish forests, cities and landscapes has largely driven a number of research projects focused on the infection biology and disease dynamics, host and pathogen genetics, the ecological role of the pathogen in its native environment, and disease control aimed at conserving and sustainably managing ash for the future.

Figure 1 A) Symptoms of ash dieback: *a)* bark canker; *b)* leaf and shoot wilting; *c)* shoot, branch and stem dieback; *d)* epicormic shoot necrosis; *e)* stem discoloration; *f)* necrosis of rachises; and *g)* leaf necrosis. Photos: Michelle Cleary. **1B)** *a)* Pollarded ash trees in Sweden (photo: Brian Muelaner; source: https://ntpressoffice.word press.com/2013/03/26/ash-dieback-and-the-threat-to-our-cultural-trees/), *b)* Production of epicormic shoots following cutting of branches may serve as direct entry courts for infection into the main stem (photo: Bengtsson et al. 2013).

Ash in Sweden

Ash occurs mainly in central and southern Sweden and is one of the most common broadleaved trees on the islands of Öland and Gotland. The natural northern distribution range for ash is mostly delimited by the climatic boundary of *Limes Norrlandicus*, which is marking the line between boreal and temperate zones, though some ash can still be found in regions such as Värmland and Dalarna, and can survive well when planted north of this boundary. The northernmost natural population is located on the east coast near 61°25'N (Halden 1928).

In the forest, ash usually grows in small, pure stands or as an admixture to stands of other broadleaved tree species, mainly European beech (*Fagus sylvatica*), pedunculate oak (*Quercus robur*), sessile oak (*Quercus petraea*), Norway maple (*Acer plantanoides*), small-leaved lime (*Tilia cordata*), wych elm (*Ulmus glabra*) and black alder (*Alnus glutinosa*) (Diekmann 1994). Pure ash stands are rare and exist in some areas such as in the region of Skåne, in the Stockholm archipelago, around Lake Mälaren and along the river Dalälven on the east coast of Sweden. The density of ash (Figure 2) is highest on calcareous soils and rocks in the lowlands stretching across from the south from Malmö towards the northeast of southern Sweden (35% of the standing volume of ash), across the lake region northeast of Gothenburg (30%), around Stockholm (7%) and on the island of Gotland (3%).

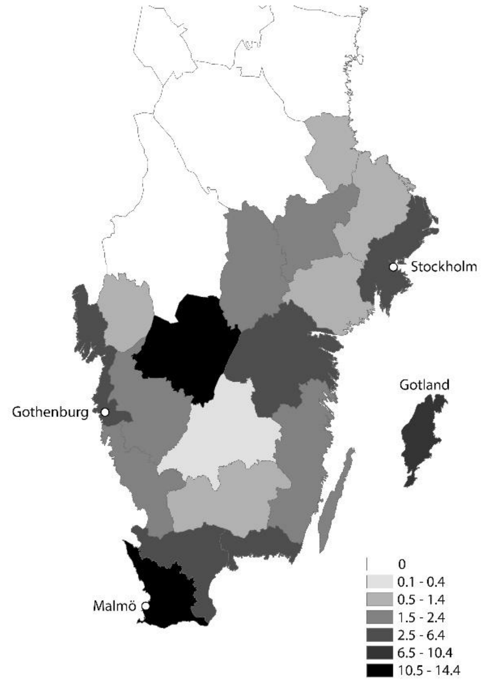


Figure 2 The standing volume of ash in Sweden on productive forest land with at least 45% broadleaved tree species. Numbers indicate the average standing volume (m^3·ha^{-1}) during 2005-2009 (NFI data).

Sample-based national forest inventories (NFIs) indicate a gradual increase in the standing volume of broadleaved trees species, including ash, since the mid-1950s (Anonymous 2015). The steady increase is due to improved silviculture and a gradual shift in the age (size) class distribution towards older stands. Ash makes up only 0.16% of the total standing timber volume in Sweden (Anonymous 2015), but approximately 7% of the standing volume of noble hardwood timber (including oak, beech, Norway maple, elm, lime, hornbeam (*Carpinus betulus*), cherry (*Prunus avium*) and ash). The total volume of ash on forest land is estimated at about 6.1 million m^3 (Anonymous 2015). Approximately half the volume includes ash with a diameter at breast height (dbh) of at least 29 cm (Skogsstyrelsen 2014). The volume of ash outside of the forest, i.e. on agricultural land, in the open landscape (meadows and pastures) and in urban areas (commonly planted along avenues and city roads), has been estimated at in excess of 10^6 m^3. The standing volume of ash peaked during 2005-2009 (Figure 3). The subsequent decline is probably due to additional cuttings in stands affected by ash dieback. Based on unpublished data from the NFI during 2010-2014, it can be estimated that ash was present in forest stands covering a total area of approximately 125,000 ha (corresponding to approx. 45 sample plots per year), with 21,500 ha having at least 30% ash, and 8,500 ha having at least 70% ash in the stand.

Disease history and surveys of damage in Sweden

Ash dieback symptoms were observed already in 2001 in Sweden (Barklund 2005; Johansson et al. 2009), and at that time was known only in a few places, though the causal agent of disease was unknown. Within just a few years, the occurrence of ash dieback was reported on trees of all age classes throughout the whole of its natural range in Sweden. Isolation and culturing work from diseased tissue, including leaves with early symptoms of infection was carried out, and the most frequently isolated taxa were included in inoculation experiments in the field during the period 2004-2006 (Bakys et al. 2009a). For the most aggressive species, which later turned out to be *H. fraxineus*, greenhouse experiments were performed to verify Koch's postulate in 2006 (Bakys et al. 2009b).

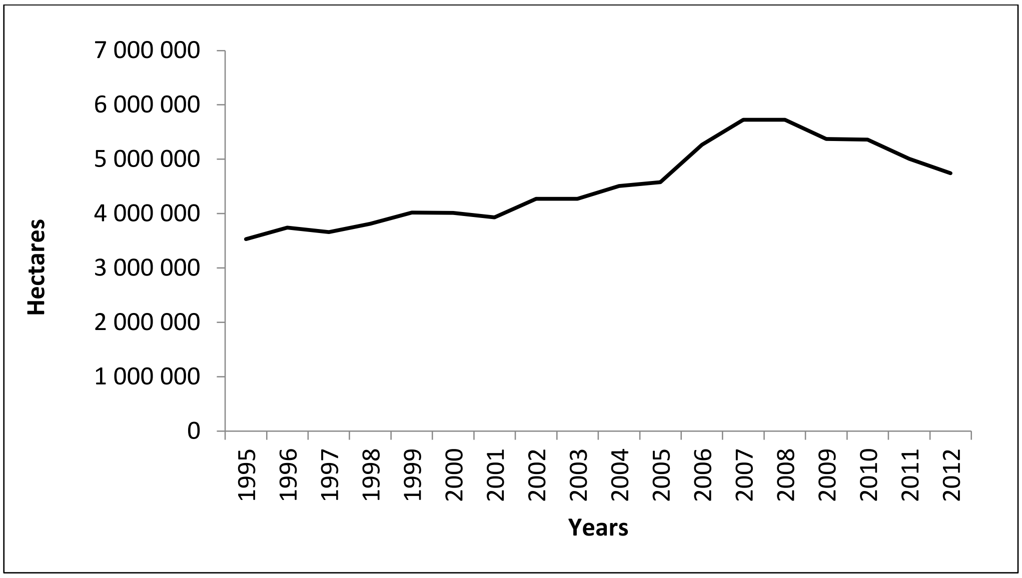


Figure 3 Total standing wood volume of ash in Sweden on productive forest land (NFI data, five-year moving average for mid-period years).

By 2009, about one-fourth of the ash trees in southern Sweden were reported as dead or severely damaged (Fischer et al. 2010). Based on unpublished data from the NFI there was a gradual increase in the extent of trees damaged by ash dieback from 15% in 2007 to 22% in 2009-2010 and a decline to 17% in 2011 (rated as >25% crown damage by visual assessment during the summer period). Inventories conducted in the region of Götaland during 2009 and 2010 indicated that approximately 30% of the ash trees (>10 cm dbh) were either severely damaged or had already died (Wulff and Hansson 2011). Similarly, surveys of veteran ash trees in the province of Västra Götaland between 2009 and 2013 showed an increase in disease severity (Bengtsson 2014).

Research on the infection biology and disease development on trees has been conducted in Sweden (Cleary et al. 2013; Bengtsson et al. 2014). Early infection stages were demonstrated using controlled infections with seedlings grown in plastic tents with relatively high humidity. One-year-old ash rachises of *H. fraxineus* covered with pseudosclerotia were placed on moist filter paper beneath the plants that subsequently produced ascocarps and sporulated. First symptoms occurred on plants two weeks after the first mature ascocarps developed. Scanning electron microscopy showed that spore attachment to leaf surfaces was associated with amorphous mucilage. Appressoria were formed and the surface wax appeared to be structurally modified and infection cushions consisting of hyphal aggregates were frequently observed. Lesions subsequently developed along the veins of the leaves characteristic to that observed in nature (Cleary et al. 2013)

Examinations of 324 natural lesions on ash shoots, branches and stems during a 3-year period showed that most active lesions grew fastest during summer; the maximum percentage of active lesions being detected in May-June and the maximum expansion rate during July-August. The high growth rate during the warmest months in Sweden correlates well with the temperature optimum (20-25^{o} C in culture) for the fungus (Kowalski and Bartnik 2010). However, some lesions can still be active throughout the remainder of the year, even at low temperatures (below 0^{o} C). This indicates that lesion expansion is a complex process, possibly including chemical or physical reactions. Of the surveyed lesions, about 20% stopped expanding each year. The recruitment of new lesions was also followed at one site. The majority of new lesions occurred at leaf scars, although a substantial number were observed without this association to leaf scars at other locations along the twig or branch. The dynamics of local epidemics indicate a potential for recovery from the disease in the event that new infections could be minimized following unfavorable environmental conditions or management strategies. Of the studied population of lesions, 25 individual lesions were genotyped using microsatellite markers. The results showed that 20% of the lesions contained more than one genotype, indicating a potential for mating and formation of ascocarps directly on infected twigs or branches, which has obviously been seen in other studies (e.g. Kirisits et al. 2012). However, no such sporulation was observed during the Swedish study (Bengtsson et al. 2014).

Studies on the foliar fungal assembly in *Fraxinus* species

Foliar fungal assemblages associated with asymptomatic *Fraxinus mandshurica* from eastern Siberia, namely from the Primorsky Krai region, were revealed using 454 sequencing technology (Cleary et al. 2016). Fungi associated with *F. mandshurica* were largely similar to those having been reported for *F. excelsior* in Europe, with the exception of a *Mycosphaerella* sp. that was common in Siberia. *H. fraxineus* was detected in 33% of samples, suggesting that in its original habitat *H. fraxineus* behaves as an endophyte on trees, without causing any apparent symptoms of dieback, or as a quiescent, latent pathogen (Cleary et al. 2016).

Economic importance of ash in Sweden

Ash is an important species among the noble broadleaved trees available to forest management in southern Sweden. The annual consumption of ash timber in the wood processing industry in Sweden amounted to 12,000 – 15,000 m^3 solid under-bark wood by 2004, with 10-30% of this volume being imported from other countries (Nylinder et al. 2007). The estimate excludes wood for energy. Ash wood is highly valued for its use in flooring, interiors, tools and furniture because of its strength and versatile properties. Throughout Sweden, many ash trees have died as a result of the chronic and cumulative effect of infections by *H. fraxineus* resulting in a loss of tree vitality, impaired growth and wood quality. Forest owners may incur financial losses depending on the extent of damage and the loss of timber volume due to ash dieback. In addition, ash timber prices can be lower, which often results when large quantities of timber enter the market, for example because of salvage cutting. Only few industries in Sweden will buy ash timber and the local use of ash for tool handles and other implements has vanished. Thus, the economic importance of ash in Sweden is consequently very limited at the national level, but may be significant for individuals and private landowners, particularly in regions where ash is a key component in hardwood forestry.

Historical and cultural significance of ash in Sweden

Ash has high cultural heritage value. Ash has traditionally been referred to as the king of trees in Sweden because it "arrives last and leaves first", i.e. its leaves flush last in the spring and fall first in the autumn. Historically, many ash trees were part of agro-forestry systems with cattle grazing, coppicing, pollarding, or combinations thereof. In several areas, ash is still being managed in such systems, e.g. pollarding, but now mainly for aesthetical reasons. Along with solitary ash, such trees can contribute significantly to the landscape at a local level. In southern Sweden, it is still common to find veteran pollarded ash trees in the landscape (Figure 1B, a). Municipalities in Sweden have invested in pollarding trees and restoration of old lapsed pollards (i.e. those that have not been pollarded for more than 30 years) as a way of maintaining cultural awareness and historical landscape features. Veteran pollarded trees are culturally significant, aesthetically pleasing, and have high biodiversity value as they are associated with many species of animals, plants and fungi. For example, pollarded trees that gradually develop stem holes left by dead branches and wood decay organisms can benefit certain birds, insects and bats. In light of the current situation with ash dieback, the consequence of pollarding is still yet unclear, particularly for veteran trees in areas where ash dieback was confirmed (Eklund, 2009), though most pollarded trees look quite dire. During the branch removal process wounds are created. These wounds may serve as infection courts for fungal pathogens, and/or the pruning practice itself promotes rejuvenation of epicormic shoots that can be easily infected by *H. fraxineus* and serve as a direct and perhaps more easy infection courts to the main stem of trees (Figure 1B, b).

Ecological importance of ash in Sweden

Ash has immeasurable value for nature conservation and is an important keystone species in natural communities protected under EU regulations (European Commission 2007). In Sweden, the ecological value of ash is reflected in the number of key habitats with ash. Unpublished data from the National Board of Forestry indicate that since 1990, more than 12,500 ha of land with at least 5% ash on the area has been designated as key habitats. These areas include productive forests, conservation forests, forest fragments and land used or previously used for agro-forestry.

Ash is very important for biodiversity. The bark is nutrient-rich, has a high pH, and creates an ideal micro-niche for mosses, fungi and insects. In 2010, ash was among the first tree species, together with elm, that was added to the Swedish Red-List (Artdatabanken, Swedish Species Information Centre, www.artdata.slu.se), and its status has recently been elevated from vulnerable to critically endangered, considered to be at high risk of extinction in the wild.

A large number (483) of species depend on ash; including 260 invertebrates (211 beetles, 30 butterflies, 9 *Diptera* sp., 5 grasshoppers and 1 snail), 87 lichens, 71 bryophytes and 65 fungi (Artdatabanken) (Hultberg et al., *manuscript in prep*). Of these, 123 are threatened (having status of either vulnerable, endangered or critically endangered). Among the species highly associated to ash, e.g. *Agrilus convexicollis*, *Tetrops starkii*, *Pyrenula nitidella*, *Perenniporia fraxinea*, and *Euphydryas maturna* (Hultberg et al. *manuscript in prep*). A survey in Sweden found ash to host an average 0.7 red-listed lichens per tree (Thor et al. 2010). In a study of the epiphytic moss *Neckera pennata,* its presence decreased with increasing mortality of fallen ash trees (Roberge et al. 2011).

These case studies suggest that with the loss of ash, the ecological cost to biodiversity will be devastating on many levels, not just in forests but wherever ash trees are found. The overall ecological impact will be generally higher for those species that have small populations and that have high- or obligate associations to ash (see also Mitchell et al. 2014). The implication over time suggests that with disease intensification and increased tree mortality, much of the biodiversity associated with ash may also disappear and reduce the diversity of species dependent on ash (Jönsson and Thor 2012).

Population genetics of the pathogen *Hymenoscyphus fraxineus*

The genetic variation in European populations of *H. fraxineus* and its non-pathogenic relative *H. albidus* was studied using fungal isolates collected from Austria, Lithuania, Denmark, Sweden and Norway (*H. albidus* only) (Bengtsson et al. 2012). *H. albidus* is a native, non-aggressive saprophyte that is morphologically similar to, and occupies an overlapping ecological niche as, *H. fraxineus*, and was the source of early confusion in the identification of the causal agent of the disease since *H. albidus* was known to exist in Europe since 1851. Utilizing seven newly developed microsatellite loci in *H. fraxineus*, the results showed that there was a very limited number of alleles (mostly two) in *H. fraxineus.* In *H. albidus,* only four of the loci produced PCR products using the microsatellite primers, and the loci were monomorphic. Arbitrary primer PCR (AP-PCR) using the M13 core sequence indicated some variation in *H. fraxineus* and no variation in *H. albidus*. The genetic variation in *H. fraxineus* was highly distributed with 99% of variation within populations; corresponding F_{ST} values were between 0.01 and 0.03. The study confirmed the genetic separation between the two *Hymenoscyphus* species and that *H. albidus* is most likely homothallic, while *H. fraxineus* is outcrossing. The limited number of alleles in *H. fraxineus*, some with quite large differences in fragment size from the respective loci, indicated that the population had undergone a recent bottleneck and subsequent expansion. The low F_{ST} values in the European population pointed to very high gene flow among the populations or very large population sizes during the recent epidemics. These findings have been largely verified by studies elsewhere in Europe (Gross et al. 2014). The likelihood that *H. fraxineus* was introduced from Eastern Asia has been demonstrated by studies with source material from Japan (Gross et al. 2014) and Far East Russia (Cleary et al. 2016) based on large differences in the genetic diversity of east Asian and European populations.

Genetics of *Fraxinus excelsior* populations in Sweden

F. excelsior is a wind pollinated tree species that has a polygamous breeding system with males, females and hermaphrodites. Genetic variation of the next generation may depend on the composition of the pollen cloud and available mates. Previous studies have consequently reported that *F. excelsior* shows a high level of genetic variation within populations and low differentiation between stands (Heuertz et al. 2001 [Bulgaria], Morand et al. 2002 [France], Heuertz et al. 2004 [western and central Europe], Hebel et al. 2006, Ferrazzini et al. 2007 [north Italy], Sutherland et al. 2010 [Britain]). Populations in central and western Europe and in the British Isles displayed extensive gene flow and thus form one large deme. In contrast, populations from southeastern Europe showed more differentiation (Heuertz et al. 2004). In Sweden, populations were more similar to those in southeastern Europe than those in western and central Europe. Furthermore, in some stands in southern Sweden, mixing of western and south-eastern gene pools seemed to have occurred (FRAXIGEN 2005).

Studies have been conducted to determine how the flowering phenology of maternal trees influences the genetic variability of their progeny, and the relationship between the genetic structure of the parental stand and the variability of produced seeds (Bochenek, Wallander, et al. [manuscript in thesis]). Significant levels of inbreeding in *F. excelsior* stands have been detected in ash populations (Heuertz et al. 2001, Sutherland et al. 2010, Morand et al. 2002); inbreeding coefficient F_{IS} estimated was low but positive (Bochenek, Wallander, et al.). There was no significantly larger inbreeding of progeny in the stand having a strong genetic substructure than in the more homogenous stand. The authors suggested that non-random mating events, particularly those that relate to flowering phenological asynchrony within stands better explained the positive F_{IS} values. It may also be that higher relatedness of sampled offspring (i.e. limited number of maternal families sampled) explained the lower estimation of inbreeding in seedlings.

In Sweden, prior to the outbreak of *H. fraxineus*, stands with *F. excelsior* were naturally regenerated. Genetic diversity in seeds became important to ensure variability in the population. However, seed that originates from a small genetic pool, which may arise when there are limited numbers of available certified seed sources, may result in eventual genetic erosion. No clear guidelines exist to help certified seed orchard and forest plantation owners harvest genetically diverse planting material. The consequence of poorly defined guidelines is seed collection from a limited number of maternal trees, increased relatedness of seeds, and eventual biparental inbreeding.

Genetic diversity in the Swedish seeds that are used for commercial trade and reforestation was evaluated (Bochenek, Granberg et al. [manuscript in thesis]). Genetic analyses utilized four highly polymorphic nuclear microsatellite markers (Lefort et al. 1999; Brachet et al. 1999). From three unique sources of seeds, average allelic richness varied between 12.3 and 14.0, which is consistent with other subpopulations in Sweden and other Scandinavian populations. Allelic richness in seed was lower than that of their parental population. In contrast, at another site where only one tree was the known source of seeds, the allelic richness was 9.75 and the number of alleles in the seeds exceeded that of the parents. Only 30-60% of alleles were shared between seeds and adult trees. Currently, ash seeds are not common on the market, and may originate from neighboring countries, e.g. Denmark and Poland (Bochenek G. M., Granberg M. et al. [manuscript in thesis]).

Genetically determined tolerance in populations and individuals in Sweden

Published studies of the genetic impact of ash dieback in Sweden so far, mainly refers to one study based on material from two clonal seed orchards (Stener 2013). In 1991-1992, a total of 106 *F. excelsior* plus-trees were phenotypically selected from 27 different stands located between latitudes 55°41'N and 58°02'N in southern Sweden. Since ash dieback was first observed in Sweden in 2001 (Barklund, 2005, 2006), it is not likely that there would have been any selection for resistance to the disease. Thus, the selected trees were regarded as a representative sample of well-performing ash in southern Sweden. One-year-old grafts were produced from each plus-tree and planted out in two seed orchards using a spacing of 3.5 x 3.5 m. The Snogeholm seed orchard (55°32'N, 13°32'E) was established in 1992 and included 100 plus-tree clones with 40 to 60 ramets each, randomly distributed within 12 blocks. The Trolleholm seed orchard (55°57'N, 13°12'E) was established in 1995 and contained 106 clones with a maximum of 10 grafts per clone distributed within eight blocks.

Results were based on individual tree observations from four assessments (September 2006, August 2007, 2010 and 2011) in the Snogeholm seed orchard and from two assessments (August 2010 and 2011) in the Trolleholm seed orchard. The susceptibility to ash dieback was analyzed by classifying the damage sustained to the crown and the stem that could be reasonably attributable to natural infections of *H. fraxineus*.

Ash dieback disease was found to be strongly genotypically controlled and the genotypic variation among individuals was considerable (Figure 4). No clones were totally resistant, but some showed reduced susceptibility and retained this tolerance after six years of heavy infection pressure. The clonal response with respect to dieback damage was stable over the studied period of 6 years and also across sites (Figure 5). Furthermore, there were no indications suggesting that stands differed in susceptibility. Moreover, the study also found autumn phenology based on leaf colouration was under moderate genetic control. The genetic correlation between autumn phenology and damage was weak to moderate (r_G from 0.38 to 0.60) and positive, suggesting a tendency for susceptible clones to have a prolonged growing season.

The high heritability of tolerance, strong Age x Age correlations and weak Genotype x Environment interactions, suggested that there is good scope for breeding less susceptible trees for the future. These findings support those from similar studies conducted in Denmark and Lithuania (McKinney et al. 2011; Pliura et al. 2011). These two seed orchards will once more be assessed in August 2016 for verification of the clonal stability over time and for control of the general development of the dieback disease since 2011.

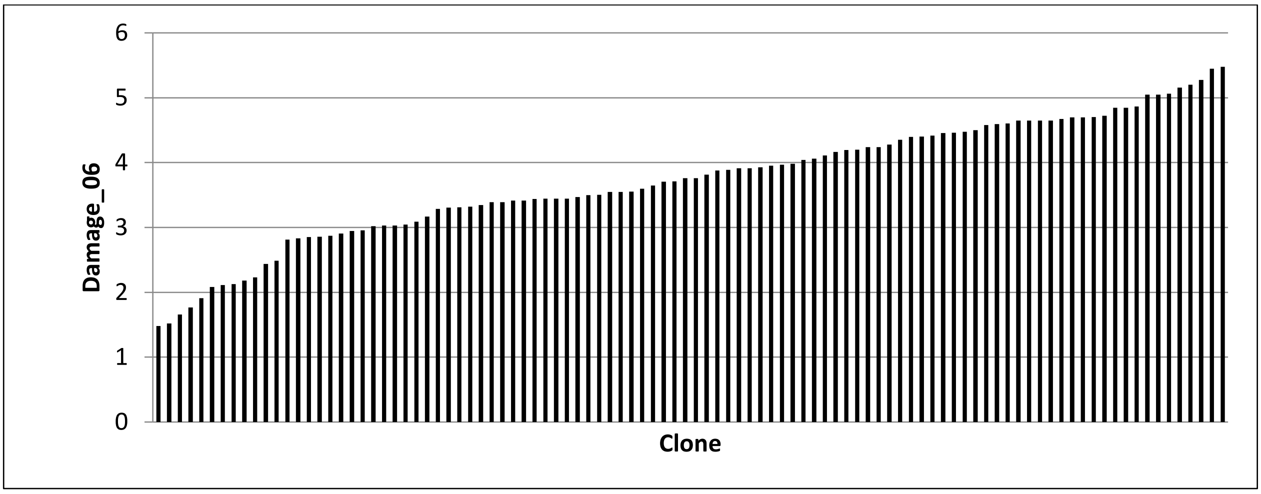


Figure 4 Genetic values via Best Linear Unbiased Prediction (BLUP) for ash dieback damage scored in year 2006 for all clones in the Snogeholm seed orchard, sorted from the least to the most damaged clone. The trait "Damage_06" was based on individual scorings of damage of the entire crown and the stem in 10 classes, from 0 (no damage) to 9 (very severe damage). (Source: Stener 2013)

The results have also led to a new project aimed at forming the basis for improvements of regeneration material of common ash in Sweden, with the emphasis on tolerance to the ash dieback disease, and at the same time establish clonal archives for gene conservation. In 2013, the Swedish University of Agricultural Sciences (SLU), in cooperation with Skogforsk, started a project in which stakeholders and the public were engaged to identify disease tolerant (vital) ash trees in forests and in the natural landscape. As a result of these efforts, selection of more than 500 vital ashes (i.e. trees without extensive crown dieback symptoms) was performed in stands seriously damaged by *H. fraxineus* across the entire natural distribution range of ash in Sweden during 2014-2015. Candidate trees will be monitored once more in about four years, after which a final selection will be performed, though during the winters of 2014-2015, scions/twigs from 109 trees were collected and used for grafting for further testing of disease tolerance. This new test population was established in a long-term field trial at Snogeholm (southern Sweden) in spring 2016.

Another ongoing project is a field test of the inheritable resistance using seedlings originating from open-pollinated seeds from 13 clones of varying susceptibility to *H. fraxineus* in the Trolleholm seed orchard (including nine which were more notably tolerant under field conditions and four with high susceptibility that were used as a comparable control). The progeny trial was established in spring 2014 in Alnarp (southern Sweden). Cumulative mortality to date ranges between 0-29% (Figure 6). Progenies from three of the ash clones (no. 114, 118, and 121) showed no mortality after two growing seasons. Similarly, the level of damage on these three clones was substantially less compared to others (Figure 7). Continued monitoring of this trial and other test populations will be important in order to make complementary selections of material to support the development of a more resistant ash population in Sweden.

Management options to alleviate the impact of ash dieback

Pre-dieback ash silviculture in Sweden

Before the introduction of the pathogen in Sweden, ash on forest sites was generally managed for commercial timber production, or to maintain biological diversity or forest aesthetics (Wahlgren 1914; Carbonnier 1947; Almgren et al. 2003; Löf et al. 2009). Overall, the management recommendations for ash in Sweden were in line with those for most other regions of Europe (Dobrowolska et al. 2011). Ash would be regenerated from natural regeneration or from planting. For commercial timber production planting would be preferred because of the better control of genetic origin, tree spacing, stand completeness, and in turn, the overall potential of trees and stand in terms of wood quality and growth capacity. Commercial stands on sites not previously stocked with ash were planted and often with trees of foreign or mixed genetic origin.

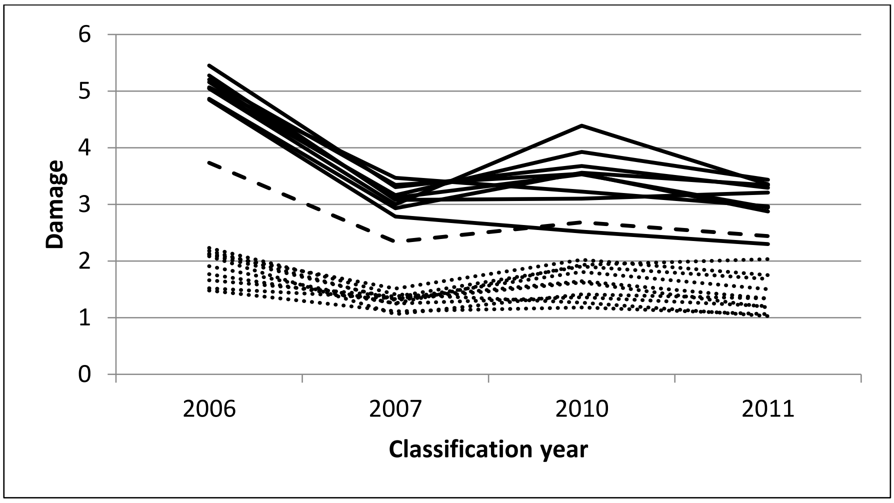


Figure 5 Genetic values via Best Linear Unbiased Prediction (BLUP) in the Snogeholm seed orchard for ash dieback damage over the classification year for the 10 most (solid lines) and 10 least (dotted lines) damaged clones. Similar results were shown for the Trolleholm seed orchard. (Source: Stener 2013)

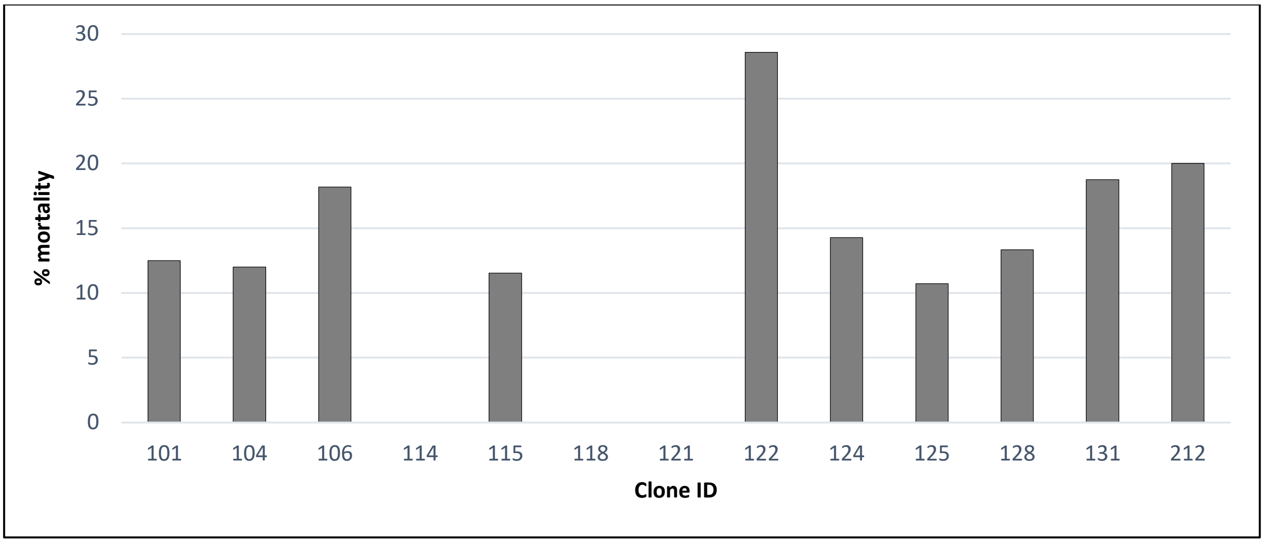


Figure 6 Cumulative mortality of progenies from 13 ash clones after two years in the Alnarp trial.

Wide spacing was recommended for young stands of ash, with re-spacing to around 2 m x 2 m already at a height of 2 m. Thinning would be initiated at a height of 6-7 m and some growers would already at this stage mark 300-400 potential future crop trees. Subsequent thinning operations would be frequent to promote these or similar trees, resulting in a final crop of 150-200 trees per ha at an age of 60-80 years. Stands on sites with high content of calcium and nitrogen would typically produce 6-7 $m^3 \cdot ha^{-1} \cdot year^{-1}$ of total wood volume. So far, there are no investigations of the impact of ash dieback on volume growth at stand level.

Post-dieback ash silviculture in Sweden

Due to a lack of understanding of the disease epidemiology and long-term implications, national recommendations for management of stands affected by ash dieback (Skovsgaard et al. 2009) were derived mainly from observations and data collected in long-term field experiments on thinning of even-aged stands of ash in Denmark. Some of these were subsequently published by Skovsgaard et al. (2010), Jakobsen (2011), Bakys et al. (2013) and Ahlberg (2014), while others remain unpublished.

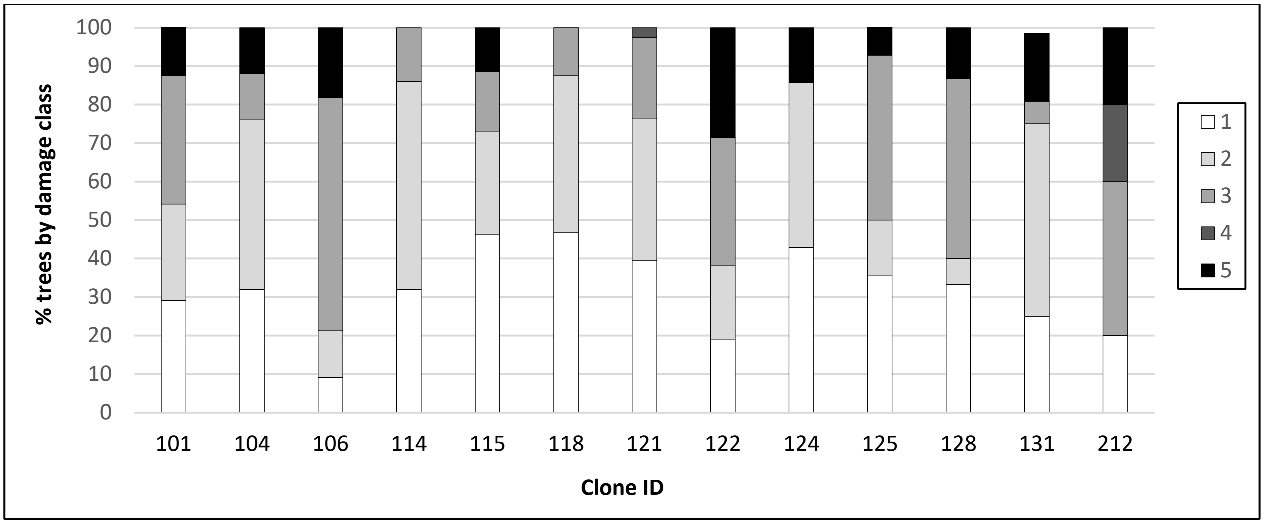


Figure 7 Damages on progenies from 13 ash clones two years after initial establishment in the Alnarp trial. Damage class ratings defined as 1, symptomless; 2, minor symptoms on shoots or leaves, AND/OR a single necrotic lesion on the shoot; 3, moderately damage; necrotic leader shoot AND 2 or 3 necrotic lateral shoots; resprouting from below the damaged shoots; 4, severely damaged; top shoot AND half of the main stem AND/OR most shoots are necrotic; trees not resprouting from below damaged shoots or resprouting from the root collar; and 5, dead. Damage class ratings were adopted from Pluria et al. (2014).

Smaller trees tended to be more severely affected by *H. fraxineus* than larger trees (Skovgaard et al. 2010). Based on that study, attacks of *Armillaria gallica* were associated with more severe crown damage while the occurrence of other damaging agents was unassociated with the dieback. In other studies, *H. fraxineus*, along with *Armillaria sp.*, have been isolated from root collars of declining ash trees (Lygis et al. 2005). It seems apparent that mixed infections of *H. fraxineus* and *Armillaria* sp. in an ash stand will result in heavier dieback symptoms than would have been the case with either of the fungal infections acting on their own.

The effect of thinning of ash stands on the severity of ash dieback has been studied (Bakys et al. 2013). The extent of crown damage increased over the growing season, though no effect of thinning intensity on disease severity was found, despite the unthinned control plots being the most severely affected. There was also a strong correlation between bud flushing phenology and dieback susceptibility; with late flushing trees being more severely affected (Bakys et al. 2013). In a separate study of disease development in seed orchards, McKinney et al. (2011) found a strong correlation between susceptibility and late senescence (though in this case, the study looked at leaf coloration as opposed to leaf shed). While it is reasonable that a prolonged attachment of leaves in the autumn would allow for extended opportunity for leaf infections to proceed through the rachises and expand into twigs and initiate branch and crown dieback, anecdotal evidence from other studies throughout Europe indicate much ambiguity with respect to spring/autumn phenology traits and disease susceptibility. Clearly more research on the role of leaf phenology and disease tolerance is warranted.

The national management recommendations for ash suffering from dieback outlines alternative silvicultural strategies depending on stand age and the severity of dieback. The overall goal of these approaches and of the differentiation depending on stand age and the extent of ash dieback is to possibly reduce the direct, as well as the derived, economic losses from ash dieback.

If the dieback is less severe, management should be conservative, avoiding unnecessary thinning interventions to maintain stand closure as much as possible. In that case it is recommended to identify and mark healthy trees. This is to avoid cutting these in error and to enable somewhat objective observations of the development of the disease over time. If the dieback is severe, it is recommended to harvest the remaining timber as soon as possible and replant the area with other suitable species.

The regeneration of stands with ash dieback should depend on the size of the area, site type, surroundings and available plant material or the potential for natural regeneration. No specific recommendations can be provided, as a proper solution depends heavily on local conditions and management objectives.

During the early 2000s, planting and re-planting of ash were discontinued almost completely. Subsequently, it soon became clear that some clones of ash are tolerant to ash dieback (see Stener 2013). However, growers still hesitate to plant ash. Since around 2010, a large number of ash stands have been cut and replanted with other species. There are no statistics available, but anecdotally some forest owners who grow ash for commercial purposes may have overcut simply to salvage fresh timber and ensure a rational approach to regeneration (minimizing regeneration costs). This seems in line with the indications from the NFI regarding the development of standing volume of ash as well as of tree health.

Conclusion

Ash in Sweden has relatively low abundance, though is highly recognized as an important species in hardwood forestry in southern Sweden and has high ecological and cultural value. Efforts are continuing to assess overall impacts of the disease. It seems that based on studies by Stener (2013) and others throughout Europe that less than 5% of the ash population is disease tolerant. In the 15 years since the detection of the pathogen in Sweden, disease intensification has led to chronic and severe damages to trees, and consequently, a decline in the ash population to the point where ash is now considered a threatened species (Artdatabanken). Efforts aimed at maintaining the genetic diversity of ash populations and the biodiversity of associated organisms by leaving healthy-looking trees on the landscape will be important in the future. Furthermore, continued work aimed at improving forest reproductive material will be critical to enable selection of targeted genotypes for further commercial propagation, breeding and future establishment of new seed orchards.

Public interest in restoration and sustainable management of ash as an important noble broadleaved tree species in Swedish forests, cities and other urban and natural landscapes is high. However, despite its high ecological and cultural importance, this public interest is also balanced with challenges for continuance of the necessary funding for tree improvement for a species with minor representation in Sweden.

References

Ahlberg, A. 2014. The influence of thinning intensity on stands of European ash (*Fraxinus excelsior* L.) affected by ash dieback – how should they be managed? A case study based on observations in young stands of ash in Denmark. SLU, MSc thesis no. 221, 63 pp.

Almgren, G., Jarnemo, L., Rydberg, D. 2003. *Våra ädla lövträd*. Skogsstyrelsen, Jönköping. 319 pp.

Anonymous 2015. Skogsdata 2015. SLU, Institutionen för skoglig resurshushållning, Umeå. 143 pp.

Bakys, R., Vasaitis, R., Barklund, P., Ihrmark, K., Stenlid, J. 2009a. Investigations concerning the role of *Chalara fraxinea* in declining *Fraxinus excelsior*. Plant Pathology. 58: 284-292.

Bakys, R., Vasaitis, R., Barklund, P., Thomsen, I.M., Stenlid, J. 2009b. Occurrence and pathogenicity of fungi in necrotic and non-symptomatic shoots of declining common ash (*Fraxinus excelsior*) in Sweden. European Journal of Forest Research. 128: 51-60.

Bakys, R., Vasaitis, R., Skovsgaard, J.P. 2013. Patterns and Severity of Crown Dieback in Young Even-Aged Stands of European Ash (*Fraxinus excelsior* L.) in Relation to Stand Density, Bud Flushing Phenotype, and Season. Plant Protection Science. 49: 120-126.

Baral, H.O., Bemmann, M. 2014. *Hymenoscyphus fraxineus* vs. *Hymenoscyphus albidus* – A comparative light microscopic study on the causal agent of European ash dieback and related follicolous, stroma-forming species. Mycology. 5: 228-290.

Barklund P. 2005. Askdöd grasserar över Syd- och Mellansverige. SkogsEko 3: 11-13

Barklund P. 2006. Okänd svamp bakom askskottsjukan. Värsta farsoten som drabbat en enskild trädart. Skogseko 3: 10-11.

Bengtsson V. 2014 Askskottsjuka - ett fortsatt hot mot våra skyddsvärda askar (Ash Dieback – a continues threat against our valuable ash, English summary), Naturvårdsenheten, rapport 2014:17.

Bengtsson, V., Stenström, A. 2009. Inventering av askskottsjuka i Västra Götalands län 2009. Länsstyrelsen i Västra Götalands län (Survey of ash dieback in the County of Västra Götaland 2009, English summary), Naturvårdsenheten, rapport 2009:80.

Bengtsson V., Stenström A., Finsberg C. 2013. The Impact of Ash Dieback on Veteran and Pollarded Trees in Sweden. The Royal Forestry Society, Quarterly Journal of Forestry 107: 27-33.

Bengtsson, V., Stenström, A., Finsberg, C. 2012. Askskottsjuka –ett nytt hot mot våra skyddsvärda träd? (Ash dieback – a new threat to our veteran trees? (English summary), Naturvårdsenheten, rapport 2012: 29.

Bengtsson, S.B.K., Vasaitis, R., Kirisits, T., Solheim, H., Stenlid, J. 2012. Population structure of *Hymenoscyphus pseudoalbidus* and its genetic relationship to *Hymenoscyphus albidus.* Fungal Ecology 5: 147-153.

Bengtsson, S.B., Barklund, P., von Bromssen, C., Stenlid, J. 2014. Seasonal pattern of lesion development in diseased *Fraxinus excelsior* infected by *Hymenoscyphus pseudoalbidus*. PLoS One, 9(4), p. e76429.

Bochenek G. M. 2011. Reproduction Biology and Population Genetics of Common Ash (F*raxinus excelsior* L) [thesis]

Bochenek G. M., Wallander E, Eriksen B. 2011. (manuscript in thesis) Seasonal variation in genetic composition of seeds in Common Ash (*Fraxinus excelsior* L.).

Bochenek G. M., Granberg M., Wallander E., Eriksen B. 2011. (manuscript in thesis) Genetic variation in Swedish seed bank of Common ash (*Fraxinus excelsior* L) compared to the population of seed origin..

Brachet, S., Jubier, M.F., Richard, M., Jung-Muller, B., Frascaria-Lacoste, N. 1999. Rapid identification of microsatellite loci using 5 ' anchored PCR in the common ash *Fraxinus excelsior*. Molecular Ecology. 8: 160-163.

Carbonnier, C. 1947. Produktionsöversikter för ask. Meddelanden från Statens Skogsforskningsinstitut 36: 1-44.

Cleary, M.R., Daniel, G., Stenlid, J. 2013. Light and scanning electron microscopy studies of the early infection stages of Hymenoscyphus pseudoalbidus on Fraxinus excelsior. Plant Pathology. 62: 1294-1301.

Cleary, M., Nguyen, D., Marčiulynienė, D., Berlin, A., Vasaitis, R., Stenlid, J. 2016. Friend or foe? Biological and ecological traits of the European ash dieback pathogen *Hymenoscyphus fraxineus* in its native environment. Scientific Reports 6, 21895; doi:10-1038/srep21895 (2016) [*published online February 22, 2016*]

Diekmann, M. 1994. Deciduous forest vegetation in boreo-nemoral Scandinavia. Acta Phytogeographica Suecica 80: 1-116.

Dobrowolska, D., S. Hein, A. Oosterbaan, S. Wagner, J. Clark, Skovsgaard, J.P. 2011. A review of European ash (*Fraxinus excelsior* L.): implications for silviculture. Forestry 84: 133-148.

Eklund, S. 2009. Hamlingav Ask, *Fraxinus excelsior*, och hur det påverkar trädets utsatthet för askskottsjukan (Pollarding of ash, *Fraxinus excelsior*, and how it influences susceptibility to ash dieback). Examensarbete Skövde Högskola.

European Commission 2007. Interpretation Manual of European Union habitats. EUR27, pp. 142. DG Environment, Nature and Biodiversity, The European Commission, Brussels, Belgium.

Fischer, R., Lorenz, M., Granke, O., Mues, V., lost, S., van Dobben, H., Reinds, G.J., deVries, W. 2010. Forest Condition in Europe. 2010 Technical Report of ICP Forests. Institute for World Forestry, Hamburg, Germany.

Ferrazzini, D., Monteleone, I., Belletti, P. 2007. Genetic variability and divergence among Italian populations of common ash (*Fraxinus excelsior* L.). Annals of Forest Science, 64: 159-168.

FRAXIGEN. 2005. Ash species in Europe: biological characteristics and practical guidelines for sustainable use. Oxford Forestry Institute, University of Oxford, UK.

Gross, A., Hosoya, T., Queloz, V. 2014. Population structure of the invasive forest pathogen *Hymenoscyphus psuedoalbidus*. Molecular Ecology. 23: 2943-60.

Hebel, I., Haas, R. & Dounavi, A. 2006. Genetic variation of common ash (*Fraxinus excelsior* L.) populations from provenance regions in southern Germany by using nuclear and chloroplast microsatellites. Silvae Genetica, 55, 38-44.

Halden, B. 1928. Asken (*Fraxinus excelsior* L.) vid sin svenskanordgräns. Svenska Skogsvårdsföreningens Tidskrift 26: 846–934.

Heuertz, M., Hausman, J.F., Hardy, O.J., Vendramin, G.G., Frascaria-Lacoste, N., Vekemans, X. 2004. Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southeastern European populations of the common ash (*Fraxinus excelsior* L.). Evolution, 58, 976-988.

Heuertz, M., Vekemans, X., Hausman, J.F., Palada, M., Hardy, O.J. 2003. Estimating seed vs. pollen dispersal from spatial genetic structure in the common ash. Molecular Ecology, 12: 2483-2495.

Hultberg, T., Sandström, J., Öhman, K., Witzell, J., Brunet, J., Rönnberg, J., Cleary, M. 2016. Resilient temperate broadleaved forests after ash dieback: can ash-associated species survive in a landscape without ash? Biological Conservation. [*manuscript in preparation*]

Höltken, A. M., Tähtinen, J., Pappinene, A. 2003. Effects of discontinuous marginal habitats on the genetic structure of common ash (*Fraxinus excelsior* L.). Silvae Genetica 52: 206-212.

Johansson, S., Stenlid, J., Barklund, S., Vasaitis, R. 2009. *Svampen bakom askskottsjukan : biologi och genetik*. (Fakta Skog, Nr. 03). Uppsala: Sveriges lantbruksuniversitet.

Jakobsen, N.A. 2011. Asketoptørre. Kronens udseende på angrebne og ikke angrebne træer ved forskellige tyndingsstyrker. University of Copenhagen, BSc thesis, 54 pp.

Jönsson, M.T., Thor, G. 2012. Estimating coextinction risks from epidemic tree death: affiliate lichen communities among diseased host tree populations of *Fraxinus excelsior.* PLoS ONE 7(9): e45701. doi:10.1371/journal.pone.0045701

Juodvalkis, A. Vasiliauskas, A. 2002. The extent and possible causes of dieback of ash stands in Lithuania. LZUU Mokslo Darbai, Biomedicinos Mokslai 56: 17.

Kirisits, T., Kritsch, P., Kräutler, K., Matlakova, M., Halmschlager, E. 2012. Ash dieback associated with *Hymenoscyphus pseudoalbidus* in forest nurseries in Austria. Journal for Agricultural Extension and Rural Development. 4: 230-235.

Kowalski, T., Bartnik, C. 2010. Morphological variation in colonies of *Chalara fraxinea* isolated from ash (*Fraxinus excelsior* L.) stems with symptoms of dieback and effects of temperature on colony growth and structure." Acta Agrobotanica 63: 99-106.

Lefort, F., Brachet, S., Frascaria-Lacoste, N., Edwards, K.J., Douglas, G.C. 1999. Identification and characterization of microsatellite loci in ash (*Fraxinus excelsior* L.) and their conservation in the olive family (Oleaceae). Molecular Ecology, 8, 1088-1090.

Lygis, V., Bakys, R., Gustiene, A., Burokiene, D., Matelis, A., Vasaitis, R. 2014. Forest self-regeneration following clear-felling of dieback-affected *Fraxinus excelsior*: focus on ash. European Journal of Forest Research, 133: 501-510

Löf, M., E. Møller-Madsen, Rytter, L. 2009. Skötsel av ädellövskog. Skogsskötselserien 10: 1-60.

McKinney, L.V., Nielsen, L.R., Hansen, J.K., Kjaer, E.D. 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (Oleraceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. Heredity. 106: 788-797.

Mitchell, R.J., Beaton, J.K, Bellamy, P.E., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Gimona, A., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Iason, G.R., Kerr, G., Littlewood, N.A., Newey, S., Potts, J.M., Pozsgai, G., Ray, D., Sim, D.A., Stockan, J.A., Taylor, A.F.S., Woodward. S. 2014. Ash dieback in the UK: A review of the ecological and conservation implications and potential management options. Biological Conservation 175: 95-109.

Morand-Prieur, M.E., Raquin, C., Shykoff, J.A., Frascaria-Lacoste, N. 2003. Males outcompete hermaphrodites for seed siring success in controlled crosses in the polygamous *Fraxinus excelsior* (Oleaceae). American Journal of Botany, 90, 949- 953.

Nylinder, M., Woxblom L., Fryk, H. 2007. Ädellöv – virke och förädling. SLU, Institutionen för Skogens Produkter, Uppsala. 194 pp.

Pautasso, M., Aas, G., Queloz, V., Holdenrieder, O. 2013. European ash (*Fraxinus excelsior*) dieback – a conservation biology challenge. Review Biological Conservation 158: 37–49.

Pihlgren, A., Hallingbäck, T., Aronsson, M., Dahlberg, A., Edqvist, M., Johansson, G., Krikorev, M., Thor, G. 2010. Var femte växt och svamp är med på nya rödlistan. Svensk Botanisk Tidskrift, 104. 212.

Pliura, A., Marciulyniene, D., Bakys, R., Suchockas, V. 2014. Dynamics of genetic resistance to *Hymenoscyphus pseudoalbidus* in juvenile *Fraxinus excelsior* Clones. Baltic Forestry 20: 10-27.

Przybył, K. 2002. Fungi associated with necrotic apical parts of *Fraxinus excelsior* shoots. Forest Pathology. 32: 387-394.

Queloz, V., Grunig, C.R., Berndt, R., Kowalski, T., Sieber, T.N., Holdenrieder, O. 2011. Cryptic speciation in *Hymenoscyphus albidus*. Forest Pathology, 41: 133-142.

Roberge, J.-M., Bengtsson, S.B.K., Wulff, S., Snäll T. 2011. Edge creation and tree dieback influence the patch-tracking metapopulation dynamics of a red-listed epiphytic bryophyte. Journal of Applied Ecology 48(3): 650-658.

Skogsstyrelsen 2013. Ask och askskottsjukan i Sverige. (Meddelande 4). Jönköping.

Skogsstyrelsen 2014. Swedish Statistical Yearbook of Forestry: Skogsstyrelsen.

Skovsgaard, J.P., Thomsen, I.M., Barklund, P. 2009. Skötsel av bestånd med askskottsjuka. Fakta Skog 2009/13: 1-4.

Skovsgaard, J.P., Thomsen, I.M., Skovgaard, I.M., Martinussen, T. 2010. Associations among symptoms of dieback in even-aged stands of ash (*Fraxinus excelsior* L.). Forest Pathology, 40:. 7-18.

Stener, L-G. 2013. Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* L. in southern Sweden. Scandinavian Journal of Forest Research. 28: 205-216.

Sutherland, B.G., Belaj, A., Nier, S., Cottrell, J.E., Vaughan, S.P., Hubert, J. & Russell, K. 2010. Molecular biodiversity and population structure in common ash (*Fraxinus excelsior* L.) in Britain: implications for conservation. Molecular Ecology, 19: 2196-2211.

Thor, G., Johansson, P., Jonsson, M.T. 2010. Lichen diversity and red- listed lichen species relationships with tree species and diameter in wooded meadows. Biodiversity and Conservation, 19: 2307.

Vitasse, Y., Delzon, S., Dufrene, E., Pontailler, J.Y., Louvet, J.M., Kremer, A., Michalet, R. 2009. Leaf phenology sensitivity to temperature in European trees: do within-species populations exhibit similar responses? Agricultural and Forest Meteorology 149*:* 735–744.

Wahlgren, A. 1914: Skogsskötsel. P.A. Norstedt & Söner, Stockholm. VIII + 728 pp.

Wulff, S., Hansson, P. 2011. Askskottsjukan i Götaland- Nationell riktad skadeinventering 2009 och 2010. Umeå: Institutionen för skoglig resurshushållning, SLU.

Zhao, Y.-J., Hosoya, T., Baral, H.-O., Hosaka, K., Kakishima, M., 2013. *Hymenoscyphus pseudoalbidus*, the correct name for *Lambertella albida* reported from Japan. Mycotaxon, 122: 25-41.

Zheng, H.-D.; Zhuang, W.-Y., 2014. *Hymenoscyphus albidoides* sp. nov. and *H. pseudoalbidus* from China. Mycological Progress, 13: 625-638.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 209 – 219.

The ash and ash dieback in Slovakia

V. LONGAUEROVÁ[1]*, A. KUNCA[1], R. LONGAUER[2], M. MAĽOVÁ[1], R. LEONTOVYČ[1]

[1] *Národné lesnícke centrum, T.G. Masaryka 22, 960 92 Zvolen, Slovakai,* [2] *Lesnická a dřevařská fakulta, MENDELU, Zemědělská 3, 613 00 Brno, Czech Republic*
** Corresponding author: Valeria.Longauerova@nlcsk.org*

Abstract

All three ash species native to Europe – common, narrow-leaved and manna ash – occur in Slovakia naturally. Common ash is by far the most important one from the ecological and commercial point of view. The combined share of the "ash" in the overall forest tree species composition is 1.6 % and its growing stock is 7.1 million cubic meters. Common ash is considered a constituent species in mixed mountain and ravine forests. Together with narrow-leaved ash, it is widespread also in the riparian and riverine forest vegetation. Natural populations of common and narrow-leaved ash represent a blend of their Central- and Eastern European gene pools in Slovakia. The two phylogenetic lineages meet in central Slovakia, and their transgression zone is up to 300 km broad due to the wind-mediated gene flow by pollen (Gömöry *et. al.* 2012).

The ash dieback was first reported in eastern Slovakia in 2004 (KUNCA *et al*. 2011). By 2010, it was present in all mountain valleys of northern Slovakia and in the lowlands. It it omnipresent now, but its severity varies among regions of the country, possibly due to local climatic and site characteristics. Also related mortality is less common than in the Czech Republic and Poland. Besides the dieback, the ash has been damaged by honey fungus (mostly *Armillaria cepistipes*) and also by *Hylesinus fraxineus* at the local scale.

Surveys carried out in 4 seed orchards revealed almost two-fold differences among their component clones in the late–summer (September) defoliation attributable to *H. fraxineus*. Clones of narrow-leaved ash performed significantly better than those of common ash in the seed orchards located in the Danube lowland (LONGAUEROVÁ et. al. 2014).

Species-specific and genetic aspects of ash dieback are studied on the series of 4 field trial plots comprising 14 provenances and 42 seed orchard progenies of common and narrow-leaved ash. First results 5 years after planting show better survival and less intensive infection in the seed orchard progenies than in provenances derived from stands and seed sources. Besides it, progenies of narrow-leaved ash outperform those of common ash in the plots situated in the lowland.

Genetic resources of common ash are conserved in-situ in 9 gene reserve forests which total area is 1,320 hectares. Basic materials of forest reproductive material of common ash include 290 approved seed stands, 207 parents of families and 2 seed orchards. There are also 6 seed stands and 1 seed orchard of narrow-leaved ash.

Due to the high heritability but very low proportion of trees tolerating infection by *H. fraxineus* in current common ash populations (e.g., LOBO *et al.* 2015), clonal seed orchards composed of dieback-tolerant clones appear to be the most efficient tool for mitigation of ash dieback. Selection and testing of candidate hyposensitive clones for new ash seed orchards in Slovakia started in 2016 thanks to the financial support of the state forest company LESY Slovenskej republiky.

Key words: ash, distribution, dieback, genetic variation, seed orchards

Introduction

Forests cover 44.3% of Slovakia and their tree species composition corresponds largely with potential natural vegetation (Šmelko et al. 2008). Broadleaved species prevail (62%), and are represented mainly by beech (33%) and oaks (13.1%). The combined share of noble hardwoods, including maple, ash, elm, alder, lime and wild fruit species, is 5.1%, of which *Fraxinus* sp. alone make 1,6 % (Table 1). All three native European ash species occur in Slovakia naturally, of which narrow-leaved ash (*Fraxinus angustifolia* Vahl.) and manna ash (*Fraxinus ornus* L.) are at their northern natural distribution limits there.

Table 1 Total area and proportion of noble hardwoods in the forest tree species composition of Slovakia according to the forest management inventories 1953, 1993 and 2014 (Source: National Forest Inventory 1949-1953, Summary Forest Management Plan 1993, Summary Information on the State of Forest in Slovakia 2006-2014).

Year	*Acer*	*Fraxinus*	*Ulmus*	*Alnus*	*Tilia*	*Prunus, Sorbus, Malus, Pyrus*
1953	15,500 ha (0.9%)	15,100 ha (0.9%)	9,800 ha (0.6%)	7,800 ha (0.5%)	1,600 ha (0.10%)	850 ha (0.1%)
1993	30,150 ha (1.6%)	20,700 ha (1.1%)	950 ha* (0.05%)	12,600 ha (0.6%)	6,100 ha (0.3%)	3,900 ha (0.2%)
2014	42,000 ha (2.1%)	32,550 ha (1.6%)	995 ha (0.05%)	16,500 ha (0.8%)	8,300 ha (0.4%)	4,150 ha (0.2%)

** Elm decline due to the Dutch elm disease*

Common ash is frequent in the most of Slovakia from lowlands up to the altitude of approx. 1,000 m, with the uppermost natural occurrence at 1 250 m in central part of the country. It absents naturally in the high mountains (The Tatras and Fatra Mts.) and former floodplains of bigger rivers. The species is important constituent of several natural forest types:

- Ravine forests from xerothermous associations with *Q. pubescens* via various subtypes of *Tilieto-Aceretum* towards *Fraxineto-Aceretum* in the mountains.
- Mixed, beech dominated highland and mountain forests *Fagetum typicum* and *Abieto-Fagetum*.
- Riparian forests of *Ulmeto-Fraxinetum, Querceto-Fraxinetum* and *Fraxineto-Alnetum* in the valleys.

Narrow leaved ash has been recognized as separate native species since the mid-20th century in Slovakia (Magic 1957). Forest surveys and management plans overlooked it even until the 1990's. The species occurs in the riverine woodland belts of the Danube and Tisza (Carpathian Basin), Morava (Vienna Basin) and their tributaries (Figure 1a). Its natural range coincides there with former floodplain hardwood forests dominated by pedunculate oak, ash and elms (*Ulmeto-Fraxinetum, Querceto-Fraxinetum* (Manica & Slobodník 2008), where the mean annual air temperature is at least 8,5 °C.). Local altitudinal maximum of the species is 210 m. Spring temperature lows seems to be of of limiting importance for its successful reproduction.

Manna ash (*Fraxinus ornus*) occurs discontinuously in the western, southern and southeastern Slovakia on warm hillsides, slopes and plateaus on calcareous and volcanic parent substrates. The largest populations of manna ash occupy southern slopes and plateaus of the Slovak Karst - Aggtelek Karst at the northernmost section of the Slovakia - Hungary border, and the Burda Hills north of the entry of the Danube into Hungary (Figure 1b). The species is considered to be an understorey shrub and is not covered by the regular forest inventory.

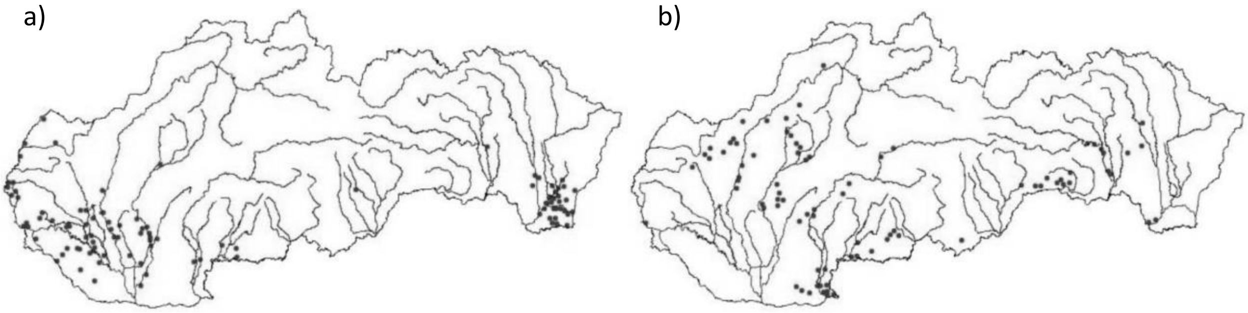


Figure 1 Natural occurrence of narrow-leaved ash (left) and manna ash (right) on their northern distribution limits in Slovakia (Source: Manica & Slobodník 2008).

A majority of local common and narrow-leaved ash populations are autochthonous and originate mostly in natural regeneration. Current distribution of common ash is close to the natural potential although its presence may have been reduced in the mountain forests due to the extensive planting of Norway spruce in the past. Like other scattered tree species, juvenile ashes suffer much of the selective browsing by ungulates. In the long-term, their chronical overpopulation may have resulted in a regress of the species in forest stands at the local level. The most recent national forest inventory (Šmelko et al. 2008) revealed, for instance, the combined share of noble hardwoods (maples, ashes and elms) as high as 21% in the established forest growths, while only 8.6 % in the subsequent thicket stage.

Among the tree species present in the forests, the common and narrow-leaved ash combined ash ranks 11th according to the area which they occupy and 9th according to the growing stock (Table 1). Their net area has increased nearly three times from 1953 to 2014, and growing stock even more steeply from 2,7 million to 6.7 million cubic meters during 30 years from 1988 to 2014. This trend is not likely to continue due to the recent ash dieback, however.

Table 2 Growing stock of common and narrow-leaved ash 1988-2014 (in million m^3 under bark) accruing to the Country Report of Slovakia for the FAO Forest Resources Assessment FRA-2015 (Moravčík et al. 2014).

Growing stock	1988	2000	2005	2014
Fraxinus sp.	2.7 mil. m^3	5.0 mil. m^3	5.8 mil. m^3	7,1 mil. m^3

Ash dieback, other diseases and pests

The first reports of severe symptoms of ash dieback come from the eastern part of Slovakia and date to 2004 (Kunca et al 2011, Figure 2A). By 2010, the dieback was spread in all mountain valleys of northern part of the country and also in the lowlands at the Danube and Tisza rivers. Timeline of the ash dieback spreading in Slovakia - following KUNCA *et al.* 2011 and more recent updates coming from the annual forest health condition reports - are presented in Figure 2A. Since 2014, infection by *Hymenoscyphus fraxineus* and ash dieback are present in the whole of the country. The dieback intensity varies considerably among the regions and mountain valleys, however. Obvious mortality attributable to the ash dieback has still not been reported from some parts of the country. This fact may be associated with the differences in local climate combined with different soils and parent substrates.

From among other fungal pathogens, particularly honey fungus *Armillaria cepistipes* Velen. was identified as a pathogen involved in a local decline of common ash on several localities in the past 2 decades. Together with *Phomopsis*, *Cytospora* and *Valsa sp.* this honey fungus species was detected on the majority of rootstocks as well as on ramets (crowns) of clones in 2 ash seed orchards we screened in 2013 (Longauerova *et al.* 2014). *Pseudomonas savastanoi* which is also known to be present in Slovakia but any significant damage on ash has not been attributed to this species since 1994. Of the insects, local overpopulation of *Hylesinus fraxinii* (Panzer, 1779) and subsequent damage of common ash was reported in 2 localities in eastern Slovakia by KUNCA *et al.* 2007. Pile traps using ash-tree sections were used successfully for the control of population density of the pest in several localities since then. Regarding manna ash, no serious health problems as well as no ash dieback have been reported in it up to now (Kunca 2011).

Knowledge of the genetic variability of common and narrow-leave ash

The both common and narrow-leaved ash are represented by a mix of their Eastern and Central-European phylogenetic lines in the territory of Slovakia. As shown by Heuertz et al. (2004), their gene pools associated with different refugia and postglacial migration routes meet in the contact zone stretching from Poland via Slovakia and Hungary to Serbia and Bosnia. Gömöry *et. al* (2012) analyzed genetic structure of 38 populations of common and narrow-leaved ash in Slovakia, Czech Republic, Poland and Hungary in 6 chloroplast haplotype (cp)DNA and 7 nuclear DNA microsatellite markers. Using maternally inherited cpDNA haplotypes, which can be spread only via seeds, they revealed a quite narrow, only 36 km wide contact zone, where ash trees from the Central-European and Eastern-European phylogenetic lineages occur mixed. In contrast to it, bi-parentally inherited nuclear gene markers, where gene flow is mediated also by pollen, revealed much wider transgression zone. Its average width is 275 kilometers. Besides it, Gömöry *et. al* (2012) detected several new haplotypes of cpDNA in addition to those detected by Heuertz et al. (2004, 2006) and Boshier et al. (2005), which suggests somehow a more complex phylogeny of common and narrow-leaved in the Western Carpathians. However, no haplotype or nuclear DNA markers for direct discrimination of common and narrow-leaved ash are known so far. The fat that the two species share also maternally inherited haplotypes of mtDNA along their migration routes, suggests their ancient hybridization in former glacial refugia in Northern Apennines, southern piedmonts of Alp and in the Balkan Peninsula (Heuertz et al. 2006).

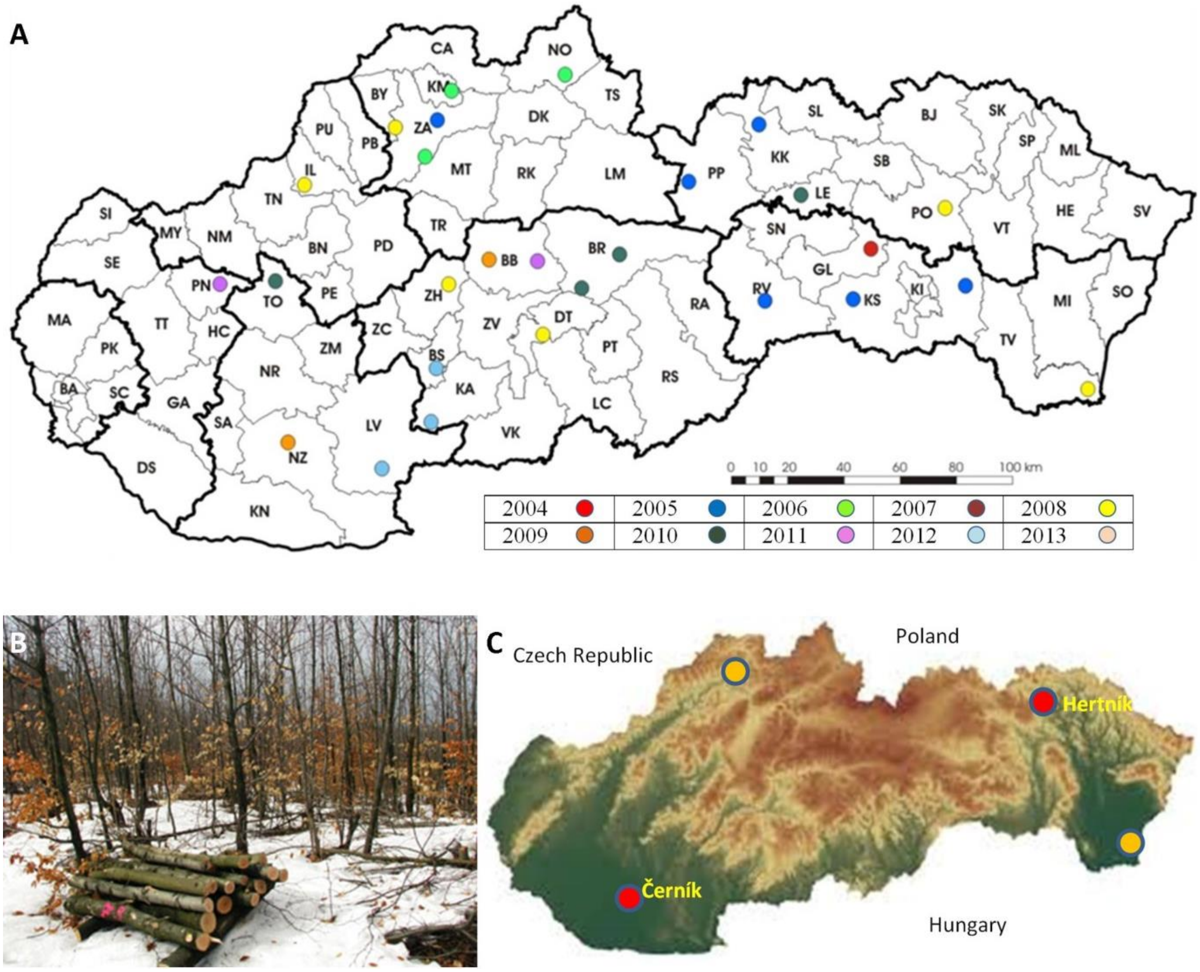


Figure 2 A) Timeline of the spatial and temporal spread of the ash dieback in Slovakia since the first reports of its presence in 2004 (Source: KUNCA *et al*. 2011, updated using more recent data). **B)** Trap-tree pile applied to control *Hylesinus fraxinii* (Kunca *et al.* 2011). **C)** Location of the two surveyed trials Černík and Hertník in the series of 4 combined progeny-provenance trial plots with common and narrow-leaved ash.

Seed orchard studies of reproductive phenology, fertility variation and potential hybridization

Bajcar (2010) studied reproductive processes in 2 clonal seed orchards of common and narrow leaved ash with focus on their effective reproductive population sizes, reproductive phenology and potential interspecific hybridization. During 2 vegetation seasons, male and female gametic contributions were estimated on the basis of fecundity and flowering phenology of 47 component clones. In order to obtain the status number as an estimator of effective population size, the author studied male and/or female fecundity of ramets in all clones:

- numbers of male and female flowers were counted in 5 inflorescences per ramet and averaged, and
- inflorescences were counted on a sample branches and converted for all crown of ramets.

Floral phenology was assessed following FRAXIGEN (2005), and scorings of pollen shedding or receptive flowers in the inflorescence were done were at intervals of 3 to 5 days.

Shares of gametes produced by individual clones were extremely unbalanced. Coefficients of variation of clonal contributions were as much as 192.8% for female flower counts and 190.1% for male flowers. Out of 47 represented clones, five most fecund ash clones produced as much has 38.7% of female and 37.7% of male gametes in the studied orchard. The effective population size was subsequently as low as 18.8 and 29.5 % of the total number of clones in the two subsequent years, mostly due the different male and female fecundities of individual clones, their reproductive phenology and pollen dispersal. At the species level, narrow-leaved ash was found producing in average twice as much male and female gametes per ramet than common ash (Machanská et al. 2014).

Only partial phenological barrier in the flowering time between the two tree species was revealed. While the peak of male flowering of common ash is approximately 10 days later than female receptivity of narrow-leaved ash, the pollen release of narrow-leaved ash and female receptivity of common ash were well synchronized in the both study years (Bajcar 2010, Machanská et al. 2013). Under the assumption no other interspecific genetic incompatibilities (as shown by Gérard et al. 2006), our results of suggest that potentially as much as 40% of hybrids present in the progeny of the studied mixed seed orchard. In addition, the majority of *F. excelsior* seeds would have originated from interspecific crossings. With reference to RAQUIN *et al* (2002) which demonstrated the possibility of hybridization of common and narrow-leaved ash in both directions by means of artificial crosses, put finding may apply to any mixed common- narrow-leaved ash population.

Altitudinal and species-specific patterns defoliation of clones in the seed orchards

In the first half of September, defoliation was scored on ramets of 108 clones in 5 seed orchards located in the lowland, hill and mountain site in western, central and eastern part of the country. Our scoring was carried out after a humid spring and second half of summer 2014. In the mountain sites, mean defoliation of clones ranged from 58 and 70%, respectively, to 100%. In the lowland seed orchard, composed of the clones of common and narrow-leaved ash (it was established in early 1990‘ies when narrow-leaved ash was still not recognized as a separate species) the overall intensity of defoliation was smaller.

1) Contrasting defoliation was revealed between common and narrow-leaved ash, because narrow-leaved ash, revealed only 32 and 65 % in comparison with 51 and 80% of common ash. Although 2-fold differences were found between the most susceptible and most tolerant clones of narrow-leaved ash, the majority of clones showed defoliation close to the mean value of 50%.

2) Condition of narrow-leaved ash clones has been significantly better in comparison with common ash (Figures 3a, 3b).

3) The extent of defoliation in different clones of common ash varied between 60-100 % (Figure 4).

Provenance trial with local common and narrow-leaved ash

The provenance trial is located in the lowland (altitude 110 m) in SW Slovakia (Trstice), approximately 50 km East of Bratislava. The trial includes 15 provenances from Hungary, Slovakia and Croatia. The trial includes 4 blocks (repeats) with 25 individuals per provenance and block. Spacing of individuals is 1 m by 3 m. We present results of this trial at the age of 14 years (Table 4).

Although the mean heights and breast height diameters do not show a clear geographic trend, all 4 provenances from the southern half of the Carpathian Basin - Central and Southern Hungary, NE Croatia - are the best growing ones. Local ash provenances showed better survival rates, on the other hand. Seemingly poorer survival of the best growing southern provenances may also be explained by competition among bigger trees already. Over all, the experiment indicates the possibility of transfer of the reproductive material of narrow-leaved ash towards the North within the Carpathian Basin.

Survival rates of Western Carpathian ash in a combined provenance and progeny experiment

Infection rate by *H. fraxineus* and proportion of vital individuals we assessed along with the current and expected final survival rate of ash in 2 parallel trial plots with ash provenances and progenies. The plots belong to a series of 4 combined progeny-provenance trial plots in different site conditions (Figure 2C). The experiment includes:

- 14 provenances of common ash covering all of its natural range in Slovakia,
- 62 seed orchard progenies of common and narrow-leaved ash from southwestern Slovakia.

Of the two plots, Černík is situated in the Danube lowland, southwest Slovakia, while Hertník in the mountain valley of northeast Slovakia close to the border with Poland. Our survey was performed at the age of 7 years in 2015, five years after outplanting, when the usual height of assessed ashes varied from 1.5 to 4 meters.

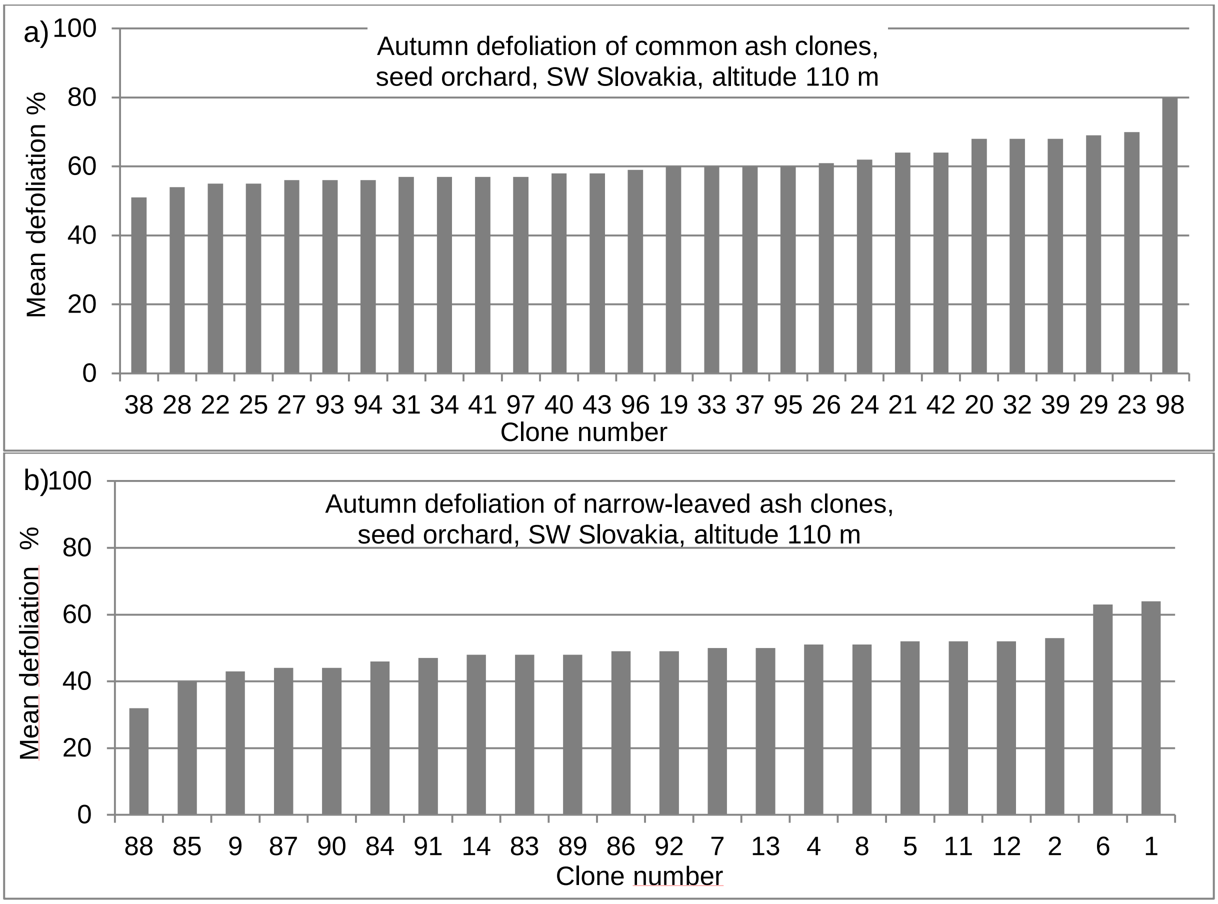


Figure 3 a and b Species-specific defoliation of common ash and narrow-leaved ash attributable to *H. fraxineus* infection in the seed orchards located in Trstice, Danube Lowland (Longauerová et al. 2014).

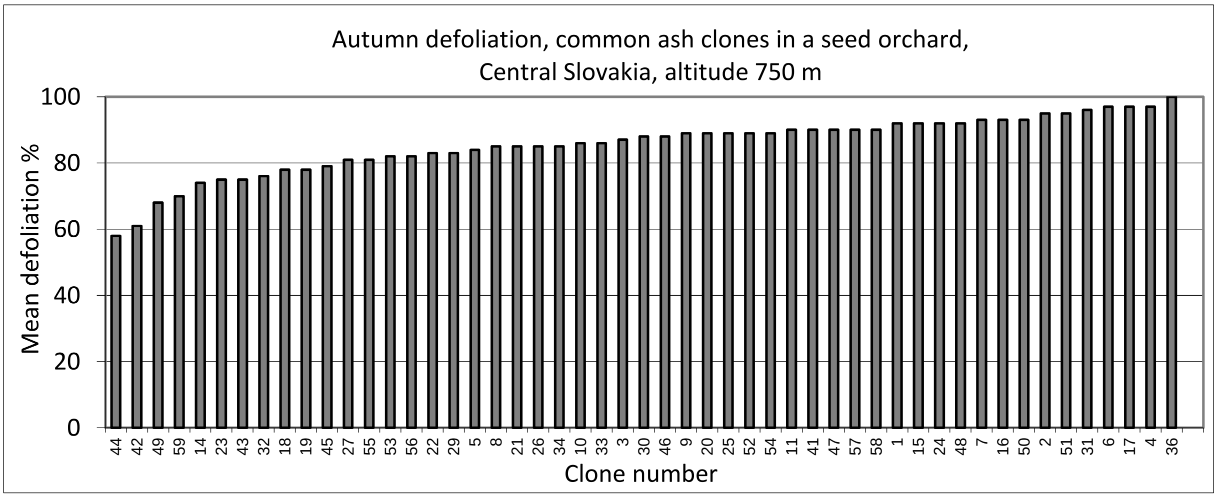


Figure 4 Mean autumn defoliation of common ash clones attributable to *H. fraxineus* in the seed orchard Zbojská, altitude 750m, in Central Slovakia (Longauerová et al. 2014).

Table 3 Mean heights, diameter and survival rates of narrow-leaved ash provenances from the Carpathian Basin in the trial plot Trstice at the age of 14 years. Local provenances are underlined, (s_x = standard deviation, FPE = *Fraxinus pensylvanica*).

	Provenance	Mean height	s_x	Mean DBH	s_x	Survival rate %
9.	Doromlás, S Hungary	9.4	2.05	8.6	3.08	67
6.	Lenes, Central Hungary	9	1.64	7.8	2.59	68
10.	Soponya, Central Hungary	8.7	1.69	7.5	2.19	76
12.	Vinkovci, NE Croatia	8.7	2.05	7.3	2.57	70
2.	Šaštín, W Slovakia	8	1.91	6.9	2.32	77
3.	V. Kapušany, SE Slovakia	7.9	1,62	6.6	3.25	50
15.	Jurová, SW Slovakia	7.8	1.64	6.4	2.39	83
1.	Tomášikovo, SW Slovakia	7.7	1.62	6,4	1,97	75
5.	Eger, N Hungary	7.7	1.72	6	2.1	67
8.	Jánkmajtis, NE Hungary	7.7	1.99	6.6	3.42	56
13.	Sládkovičovo, SW Slovakia	7.3	1.73	6.2	2.24	82
4.	Vámosatya, NE Hungary	7.2	1.81	5.8	2.39	66
7.	Sopron, NW Hungary	7.2	2.34	5.7	2.6	59
14.	Križovany n. D. SW Slovakia	6.7	1.73	4.8	1.76	82
11.	FPE Csemő, Central Hungary	6.5	1.8	5.3	2.12	77

The first results obtained 5 years after planting reveal, first of all, species-specific patterns of the survival and infection by *H. fraxineus*: In the lowland site, seed orchard progenies of narrow-leaved ash revealed better survival and less infection symptoms in comparison with provenances and orchard progenies of common ash. In the high altitude site, situated outside the natural range of narrow-leaved ash, its survival rate was poorer and infection medium in comparison with common ash.

Table 4 Current (autumn 2015) survival rate and mean ash dieback scores of 7-year old common and narrow-leaved ash originating from stands (provenances) and seed orchard.

	Species and type of tested reproductive material	Current survival rate	Mean ash dieback score *
North-West Slovakia Hertník, altitude 450 m Slope to alluvial oak-dominated woodland	*F. excelsior* – 14 provenances from forest stands	78%	3.0
	F. excelsior – 21 halfsib seed orchard progenies	90%	2.3
	F. angustifolia – 15 halfsib seed orchard progenies	83%	2.6
South-West Slovakia Černík, altitude 150 m Riparian mixed oak-dominated woodland	*F. excelsior* - 14 provenances from forest stands	58%	3.9
	F. excelsior - 21 halfsib seed orchard progenies	83%	3.1
	F. angustifolia - 21 halfsib seed orchard progenies	79%	3.0

** 6-grade scale from 1 = no infection to 6 = dead*

Comparison of the survival and infection rates revealed also a difference in relation to the quality of a seed source: seed orchard progenies, the parents of which were identified by means of individual selection of superior trees, perform better than progenies either of approved stands resulting from bulk phenotypic selection or seed sources of average phenotypic quality.

Besides the species and quality of basic material, significant site effects on the growth of common and narrow leaved ash were identified on the same material. The site effects were manifested as early as 2 years after planting by Bajcar (2011).

Table 5 Expected survival rates based on the proportion of dieback-tolerant (hyposensitive individuals) in 2 parallel field trials with identical provenances and similar sets of open-pollinated progenies of common and narrow-leaved ash, age 7 years.

	Species and tested reproductive material	Proportion of vital individuals*	Expected final survival**
S-W Slovakia, Černík, altitude 150 m Riparian mixed oak-dominated woodland	Reproductive material from stands and approved stands:		
	F. excelsior, 14 provenances	14.3%	1.1%
	Open-pollinated seed orchard progenies:		
	F. excelsior, 21 progenies	15.3%	3.1%
	F. angustifolia, 21 progenies	26.0%	3.4%
NE Slovakia, Hertník, altitude 450 m Former farmland in transition to alluvial woodland	Reproductive material from stands and approved stands:		
	F. excelsior, 14 provenances	15.7%	2.3%
	Open-pollinated seed orchard progenies:		
	F. excelsior, 21 progenies	30.9%	4.5%
	F. angustifolia, 15 progenies	13.2%	1.8%

* *Class 1 - - infection-free individuals + Class 1.5 – individuals with putative infection on leafs*

** *Class 1 only - infection-free individuals*

Conservation and utilization of the genetic resource of common and narrow-leaved ash

Ash is present largely in natural and seminatural condition in the territory of Slovakia. Some of its larger populations have been conserved in-situ in 9 gene reserve forests. Their total area is 1,320 hectares and the net proportion of ash is 7%. The concept of gene reserve forests relies largely on dynamic conservation, preferably close-to-nature silviculture supporting natural reproduction, regeneration and selection processes.

Basic materials of forest reproductive material of common ash include 290 registered seed collection stands, 2 seed orchards and 207 parents of families. Basic materials of narrow-leaved ash includes only 6 approved stands for seed collection, 1 seed orchard, and 29 parents of families.

Table 6 Sources of forest reproductive material according to the quality of their basic materials: Source identified = seed sources, no selection applied, Selected = approved seed stands resulting from mass phenotypic selection, Qualified = seed orchards composed of ramets of plus trees, individual parents of families.

	Fraxinus excelsior			*Fraxinus angustifolia*		
EU/OECD Category	Source-identified	Selected	Qualified	Source identified	Selected	Qualified
2015	11 stands /3.3 ha	290 approved stands/491 ha	2 seed orchards 207 parents of families	-	6 approved stands /16 ha	1 seed orchard, 29 parents of families

Despite of the progressing ash dieback, relatively big numbers of seedlings and transplants are grown in forest nurseries (Table 6). Typical planting stock of ash includes bare root, 2-4 years old transplants. Following the records of official body for forest reproductive material (National Forest Centre 2015) 150-175 ths. Ash transplants have been outplanted in local forests annually.

Table 7 Planting stock of ash under production - all ages together - in forest nurseries by autumn 2015

EU/OECD Category:	Source identified	Qualified	Selected	Total
Fraxinus excelsior				
2015	287 ths.	537 ths.	48 ths.	873 ths
Fraxinus angustifolia				
2015	-	-	69 ths.	69 ths.
2014	-	33 ths.	14 ths.	47 ths.

Discussion and conclusions

There is a strong genetic control of the ash dieback (0.75-0.83) but only 1 to 5% of currently living ash trees tolerate infection by *H. fraxineus* (LOBO *et al.* 2015, Kjaer et al. 2012). If only expected number survives the present dieback, population sizes and densities of the species drop drastically over the major part of the natural range of common and likely also of narrow-leaved ash. The ash dieback would thus result in the severe genetic drift, followed by inbreeding among the progenies of surviving trees in next generations. The ash is wind-pollinated and its pollen can be transferred on considerable distances but the distance between surviving seed and pollen parents will be critically important. Majority of pollen seems to be dispersed in few hundred meters in forests stands (Frascaria-Lacoste et al., 2006). In a more open landscape, 85% of effective pollinations may occur within the distance of 100 m from studied male trees (Bacles & Ennos 2008). In addition, differences (assymetries) among individual trees in their paternal success rates were observed (Gérard et al. 2006), likely due to different flowering intensity as well as flowering time among ash trees (Machanská et al. 2013).

High genetic control of tolerance to the infection by *H. fraxineus* combined with extremely low proportion of infection-tolerant ash trees provides a strong argument for establishment of seed orchards composed of dieback-tolerant clones. Such orchards will produce infection-tolerant progenies of ash in a reasonable time (less than 10 years) for restoration of ash populations.

Of the two clonal seed orchards of common ash in Slovakia, one comprises genotypes from mountain forests of central Slovakia and one from lowlands of southwestern Slovakia. Narrow-leaved ash orchard comprises genotypes from southwestern Slovakia. Selection of plus trees was carried a long time before the ash dieback with focus on the stem quality and growth vigor. The majority of plus trees grafted in the seed orchards are still alive in forest stands. Having revisited them in 2014, we have found their current condition obviously better in comparison with the mean overall condition of local ash population. As we show in Tables 4 and 5, the health and survival rate of the seed orchard progenies is also significantly better in comparison with bulk "provenances" derived from approved stands and seed sources. Within individual seed orchards (Figure 3 and 4), only some genotypes appear to be infection tolerant, however. Hence, new round of selection and testing of hyposensitive clones for novel seed orchards started with financial support of the state forest company LESY SR from 2015. We find identification of candidate ash trees possible even under medium to moderate intensity of ash dieback in forest stands. Reliable yet financially acceptable testing of their individual tolerance to *H. fraxineus* is critically important for us, however. DNA markers appear to be still rather expensive while conventional infection tests require cloning of candidate individuals. We therefore try to standardize fast-track infection tests of branches using green or autumn current-year growths.

Regarding the reproduction, dioecy prevails in ash and effective reproductive sizes vary considerably in its populations (e.g., Bajcar 2011). Clonal seed orchards producing dieback-tolerant genotypes for ash restoration therefore need to be big enough in order to avoid the effects of genetic drift. They could comprise 50 but preferably 100 components clones.

In a search of dieback-tolerant trees, their expected proportions of 1-5% are to be found in larger natural populations. Genetic makeup of smaller ash populations is influenced by historical forest fragmentation, shifting tree species compositions as well as forest regeneration using progenies of few seed parents. National regulation requires at least 10 seed parents to be represented in seed lots of scattered tree species only from 2004.

In forest stands, preservation of as many as possible infection-tolerant individuals is critically needed. Otherwise population densities of ash decline to a level when their gene pool is likely to be impoverished by genetic drift and inbreeding in the next generation(s). The ash dieback progresses particularly rapidly in younger ages when trees are in the sapling and pole stage. Forest managers then prefer culling or complete elimination of whole ash groups. Even in the early ontogenetic stages, infection-tolerant individuals can be identified visually by the absence of crown malformations, absence of lesions and dead shoots. Having tested reliability of such identification in various periods of the year, the period of leaf flushing and beginning of shoot elongation appeared to be most suitable. After some additional testing, a technical guide for identification of perspective ash individuals in silvicultural interventions (clearing, thinning) can be developed in order to support dieback-tolerant ash individuals.

Acknowledgement

This work was supported by the Slovak Research and Development Agency based on the agreement No. APVV-0707-12 for the project "Research of Disturbance Factors Affecting Long Lasting Development of Slovak Forest Health". This work was supported by the grant of the Slovak Research and Development Agency No. APVV-0135-12 for the project "Adaptive Potential of Forest Tree Species in the Context of Climate Change". This work was supported by the Slovak Research and Development Agency under the projects no. APVV-14-0567 „Information and Warning System for Invasive Organisms in the Forests and Urban Areas".

Literature

Anonymous, 1954: Národná inventarizácia lesov 1949-1953. [National Forest Inventory 1949-1953]. SVPL, Bratislava, 345 pp. (in Slovak).

Anonymous, 1993: Súhrnný lesný hospodársky plán SR 1994. [Summary Forest Management Plan of the Slovak Republic]. Lesoprojekt, Zvolen, 270 pp. (in Slovak).

Anonymous, 2015: Súhrnné informácie o stave lesov SR 2006 – 2014 [Summary information on the state of forests in Slovakia 2006-2014], NLC-ÚLZI Zvolen, 130 p. (in Slovak).

Bacles, Ennos, R.A., 2008: Paternity analysis of pollen-mediated gene flow for *Fraxinus excelsior* L. in a chronically fragmented landscape. Heredity 101: 368-380.

Bajcar, V., Longauer, R., 2012: Poznatky o biológii, reprodukčných procesoch a genofonde jaseňa štihleho *Fraxinus excelsior* a jaseňa úzkolistého *Fraxinus angustifolia* Vahl. [Knowledge of the biology, reproduction and gene pool of common ash *Fraxinus excelsior* and narrow-leaved ash *Fraxinus angustifolia* Vahl.]. *Lesnícky časopis - Forestry Journal* 583:131-189 (in Slovak).

Bajcar, V., 2010: Reprodukčné procesy a testy potomstiev jaseňa štíhlého a úzkolistého [Reproduction processes and progeny tests of common and narrow leaved ash]. PhD Thesis. Faculty of Forestry, Technical Uniersity in Zvolen, 152 pp. (in Slovak).

Douglas, G., Pliura, A., Dufour, J., Mertens, P., Jacques, D., Fernandez-Manjares, J., Buiteveld, J., Parnuta, G., Tudoroiu, M., Curnel, Y., Thomasset, M., Jensen, V., Knedsen, M.A., Foffová, E., Chandelier, A., Steenackers, M., 2013: Common ash (*Fraxinus excelsior* L.). In: Paques, L.E. (ed.): Forest Tree Breeding in Europe. Current State-of-the-Art and Perspectives. Managing Forest Ecosystems 25, Springer Dordrechtt-Heidelberg-New York-London, p. 403-462.

Gérard, P.R., Klein, E.K., Austerlitz, F. Fernández-Manjarréz, J.F., Frascaria- Lacoste, N. 2006: Assortative mating and differential male mating success in an ash hybrid zone population. BMC Evolutionary Biology 6: 96. http://www.biomed central.com/1471-2148/6/96

Gömöry, D., Paule, L., Krajmerová, D., Romšáková, I., Piecka, J., 2012: Gene exchange across a postglacial contact zone in *Fraxinus excelsior* L. Silvae Genetica 61(1):18-*27.*

Heuertz, M., Carnevale, S., Fineschi, S. *et al.* 2006: Chloroplast DNA phylogeography of European ashes, *Fraxinus* sp. (*Oleaceae*): roles of hybridization and life history traits. Molecular Ecology 15: p. 2131-2140.

Heuertz, M., Vekemans, X., Hausman, J.-F., Palada, M., Hardy, O.J., 2003: Estimating seed vs. pollen dispersal from spatial genetic structure in the common ash. Molecular Ecology 12: 2483-2495.

Kunca, A et al., 2013: Metodické usmernenie k chronickému chradnutiu jaseňov. [Methodical guideline regarding the chronical ash dieback]. NLC-LVÚ, Lesnícka ochranárska služba, 12 s. (retrievable at http://www.los.sk/pdf/usm_jasen_13.pdf)

Kunca, A., Leontovyč, R., Zúbrik, M., Gubka, A., 2011: Bark beetle outbreak on weakened ash trees and applied control measures. EPPO Bulletin 41 (1): 11-13. doi: 10.1111/j.1365-2338.2010 .02428.x

Kunca, A., Leontovyč, R., 2011: Occurrence of Ash dieback in Slovakia since 2004. In: DELB., H., PONTUALI, S. (eds): Biotic Risks and Climate Change in Forests. Proceedings from the 10th IUFRO Workshop of WP 7.03.10 „Methodology of Forest Insect and Disease Survey in Central Europe". Fakultät für Forst- und Umweltwissenschaften der Albert-Ludwigs-Universität Freiburg and Forstliche Versuchs- und Forschungsanstalt (FVA) Baden-Würtenberg, p. 170-171.

Kunca, A. (ed.), 2006: Výskyt škodlivých činiteľov v lesoch Slovenska za rok 2005 a ich prognóza na rok 2006. [Occurrence of pest agents in Slovak forests in 2005 and the prognosis for 2006]. Lesnícky výskumný ústav, Zvolen, 15 pp. (in Slovak).

Longauerová V., Maľová M., Kunca A., Leontovyč R. 2013: Poznatky z hynutia jaseňov spôsobovaného hubou *Hymenoscyphus pseudoalbidus* (ana. *Chalara fraxinea*). [Knowledge of the ash dieback caused by fungus *Hymenoscyphus pseudoalbidus* (ana. *Chalara fraxinea*)]. *In*: KUNCA, A. (*ed.*), Aktuálne problémy v ochrane lesa 2013, Zborník referátov z 22. medzinárodnej konferencie. Národné lesnícke centrum, Zvolen, p. 149-152 (in Slovak).

Longauerová, V., Maľová, M., Adamčíková, K., Kunca, A., Kadási-Horáková, M., Pastirčáková, K., 2015: Zdravotný stav jaseňov v semenných sadoch Slovenska v súvislosti s chradnutím vplyvom *Chalara fraxinea* (*Hymenoscyphus fraxineus*). [Health condition of ash in the Slovak seed orchards in relation to ash dieback caused by *Chalara fraxinea* (*Hymenoscyphus fraxineus*)]. *In*: Kunca, A. (*ed*.), Aktuálne problémy v ochrane lesa 2015, Zborník referátov z 24. medzinárodnej konferencie. Národné lesnícke centrum, Zvolen, s. 78-82 (in Slovak).

Longauer, R., Hoffmann, J., 1996: Noble hardwoods in Slovakia. Country Report. *In:* Turok, J., Eriksson, G., Kleinschmitt, J., Canger, S., (*eds.*) Noble Hardwoods Network. Report of the first meeting 24-27 March 1996, Escherode, Germany. International Plant Genetic Resources Institute, Rome, Italy. p. 73-79.

Longauerová, V., Rozsypálek, J., Papić, S., Longauer, R., 2015: Chradnutie jaseňov, súčasný stav a praktické aspekty [Ash dieback, current state and practical aspects]. *In*: Štefančík, I., Bednárová, D. (*eds*.): Aktuálne problémy v zakladaní a pestovaní lesa 2015. Zborník referátov z 3. medzin. konferencie 5.-6. októbra 2015 v L. Hrádku. Národné lesnícke centrum - Lesnícky výskumný ústav Zvolen, s. 82-91 (in Slovak).

Magic, D. 1957: Jaseň úzkolistý (*Fraxinus angustifolia* Vahl.) na Slovensku [Narrow-leaved ash (*Fraxinus angustifolia* Vahl.) in Slovakia]. *Lesnícky časopis* 4(3): p. 269-291 (in Slovak).

Machanská, E., Bajcar, V., Longauer, R., Gömöry, D., 2013: Effective population size estimation in seed orchards: A case study of *Pinus nigra* ARNOLD and *Fraxinus excelsior* L. /*F. angustifolia* VAHL. Genetika 45(2): 575-588.

Manica, M., Slobodníik, B. 2008: Poznámky k výskytu a rozšíreniu jaseňov (*Fraxinus* L.) na Slovensku. Acta Facultatis Ecologiae 18: 35–46 (in Slovak).

Moravčík, M., Kovalčík, M., Schwarz, M., Konôpka, B., 2014: FAO Global Forest Resources Assessment 2015 - Country Report Slovakia, Rome, 2014, 96 pp. (retrievable at http://www.fao.org/3/a-az196e.pdf)"

Papić, S., Rozsypálek, J., Longauer, R., 2015: Ash dieback – a review and silvicultural aspects. In: Houšková, K., Černý, J. (eds): Proceedings of Central European Silviculture. Mendelova univerzita v Brně, Lesnická a dřevařskáfakulta, p.198-207.(retrievable at http://fraxinus.mendelu.cz/uzpl/index.php/download/category/40-2015)

Pliūra, A., Heuertz, M. 2003: EUFORGEN Technical Guidelines for genetic conservation and use for common ash (*Fraxinus excelsior*). International Plant Genetic Resources Institute, Rome, Italy: 6 pp.

Slobodník, B., Čaňová, I., Paule, L. 2006: Fenológia kvitnutia a olisťovania dvoch druhov mediteránnych jaseňov (*Fraxinus* L.) na severnej hranici ich rozšírenia. [Flowering phenology and leaf development in two ash mediterranean ash (*Fraxinus* L.) species at their northern distribution limit]. *In:* Rožnovský, J., Litschmann, T., Vyskot, I. (ed.): Fenologická odezva proměnlivosti podnebí, Brno 22.3.2006, CD ROM. (http://www.cbks.cz/sborn%C3%ADk06 /prispevky/prispevky.htm)

Šmelko, Š., Šebeň, V., Merganič, J., Jankovič, J., 2008: Národná inventarizácia a monitoring lesov SR 2005-2006. Základná koncepcia a výber zo súhrnných informácií. [National forest inventory of the Slovak Republic 2005-2006. Basic concept and selected summary results]. Národné lesnícke centrum – Lesnícky výskumný ústav Zvolen, 15 pp. (in Slovak).

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 220 – 227.

The current situation concerning severity and causes of ash dieback in Ukraine caused by *Hymenoscyphus fraxineus*

K. DAVYDENKO[1]*, V. MESHKOVA[2]

[1] Kharkiv State Zooveterinary Academy, Mala Danilovka, Dergachi region, 62341, Kharkiv, Ukraine, [2] Ukrainian Research Institute of Forestry & Forest Melioration, Pushkinska st. 86, 61024 Kharkiv, Ukraine
** Corresponding author: kateryna.davydenko@gmail.com*

Abstract
Dieback of European ash (*Fraxinus excelsior*), caused by the alien invasive fungus *Hymenoscyphus fraxineus*, started in the early 1990s in Poland and has spread over Europe since then. In Ukraine, this causal agent of ash dieback was isolated first in 2011 in the east and subsequently from several other locations in the west and central parts of country. Intensity of the ash dieback seems to be far severe on sites with high humidity where strong mortality of ash trees for all ages were observed in 2014- 2015. Besides *H. fraxineus* damage, fungi from genus Armillaria and bacterial cancer play an crucial role as secondary pathogens in ash decline in humid regions while the ash sawflies and other insects cause some strong additional damage in hot and dry climate regions. Hot and dry areas in the south and south-east Ukraine are probably not as suitable for ash dieback prevalence as west and north parts of Ukraine. However, we assume that the ash dieback along with secondary pathogen will also reduce ash stands significantly in Ukraine.

Keywords: ash dieback, *Hymenoscyphus fraxineus*, *Fraxinus excelsior*, Armillaria, bacterial cancer, *Pseudomonas syringae* pv.savastanoi

Introduction

Massive ash dieback caused by *Hymenoscyphus fraxineus* (Queloz et al. 2011) is an emerging invasive disease that affects mostly common ash (*Fraxinus excelsior*) stands in many European countries last decades (Pautasso et al. 2013). The typical symptoms of this disease are wilting and necroses of ash leaves and rachis, necrotic lesions on stems, twigs and shoots, gradual crown damage, wood discolouration and following mortality of ash trees (Kowalski and Holdenrieder 2009, Bakys et al. 2013). *H. fraxineus* was found on *F. excelsior* and *F. angustifolia* in Europe, *F. mandshurica* in east Russia, China, Japan, Korea and *F. rhynchophylla* in Korea (Gross et al. 2015).

In Ukraine, the stands of *F. excelsior* constitute only about 6% of all deciduous forests (Gul'chak 2012). Notwithstanding this *F. excelsior* is often among the major tree species found in parks, city plantations and forest shelterbelts and taken together has considerable socio-economic importance (Davydenko and Meshkova 2014). In eastern Ukraine, the first appearance of symptoms of ash decline wase observed in 2010 and the low frequency of *H. fraxineus* were detected in symptomatic shoots in 2012 (Davydenko et al. 2013). Although there is little information on ash decline from western Ukraine (Matsiakh and Kramarets 2014), it seems like the spread of ash decline into eastern Ukraine was relatively slow. It was suggested that the disease was in an early phase of spread in local stands of *F. excelsior*, i.e. much later than in many European countries (Davydenko et al. 2013). Incidence and severity of ash dieback varies significantly in different parts of the Ukraine and it appears to be most serious in the west of Ukraine which borders with Poland where ash dieback was found first in 1990s (Kowalski 2006). Moreover, there are still areas in South Ukraine, where the disease was not reported yet, likely because of common ash is very rare there. Apart from *F. excelsior*, dieback also occurs on narrow-leaved ash and weeping ash (Kirisits et al. 2010, Kirisits and Schwanda 2015). But no symptoms of ash dieback have been observed in Ukraine forest on green ash (*F. Lanceolata)* and *F. pennsylvanica* up to date.

Notwithstanding well-known and validated dates about distribution of the ash dieback in Europe, little information is known about status of this disease in Ukraine. The aim of our research was 1) report about the ash dieback prevalence in Ukraine up to date and to confirm presence of *H. fraxineus* by isolation from symptomatic shoots; 2) monitor severity of ash dieback over a period 2011-2015 and 3) identify other phenomena involved in ash dieback in Ukraine

Materials and methods

Field studies were carried out in the forest stands and shelter belts of 6 regions (Kharkiv, Sumy, Poltava, Luhansk, Kyiv and Zhytomyr) which are located in Steppe (Luhansk), Forest Steppe (Kharkiv, Poltava, Sumy and Kyiv) and Forest (Zhytomyr).These stands represented mixed forest stands 10 to 80 years old with dominance of *Quercus robur* and *F. excelsior*. Forest stands were selected for sampling in the beginning of summer 2011, and assessment was conducted in June and end of August at 10 temporary study plots which were established in these stands (Table 1). The data of ash trees condition were collected from mixed stands which were surveyed in 2011 -2015. The classification system that subdivides trees into six classes was applied to evaluate tree condition (Sanitary rules in the forests of Ukraine 1995). These classes were determined for all trees: 1st class – healthy; 2nd class – weakened; 3d class – strongly weakened; 4th class – drying; 5th – recently died; 6th – died over year ago). All trees in each localities were assigned at six classes. Index of tree condition for forest stand was calculated as mean weighted from trees number of each category of sanitary condition.

Table 1 List and description of inspected stands by localities.

No plot	Name of locality	Type of stand	Age of trees	Coordinate	Successful isolations of *H. fraxineus*, %
1a	Kharkiv, Polotilovka	forest	28-30	49°55'31.7"N 36°10'28.8"E	85
1b	Kharkiv, Vysokii	forest	25	49°53'48.3"N 36°14'04.9"E	90
1c	Kharkiv, Babai	forest	40	49°53'12.4"N 36°08'39.6"E	65
2a	Sumy Ahtyrka	forest	80	50°19'34.1"N 34°51'28.8"E	70
2b	Sumy Trostynets	forest	48	50°28'09.8"N 34°59'18.6"E	58
2c	Sumy	forest	55	50°59'02.6"N 35°01'48.6"E	84
3	Poltava	shelter belts	35	50°59'02.6"N 35°01'48.6"E	72
4	Kyiv	shelter belts	38-40	50°59'02.6"N 35°01'48.6"E	56
5	Zhytomyr	forest	47-50	50°59'02.6"N 35°01'48.6"E	68
6	Luhansk	forest	28-32	50°59'02.6"N 35°01'48.6"E	31

Crown damage was rated applying the following six point system for disease intensity according to the number of symptomatic shoots and crown damage (Metzler et al. 2012): (0) no symptomatic shoots and ash dieback-symptoms; (1) one or four symptomatic shoots, minor and indirect symptoms (uneven foliage expansion, necrotic lesions in healthy shoots; (2) more than 4 symptomatic shoots with necrotic lesions formation in 10 % of crown size; (3) more than 10 symptomatic shoots with necrotic lesions formation in 10–50 % of crown size; (4) more than 50 % of all shoots are symptomatic and (5) tree mortality.

Insects and fungi in the sample plots were identified by symptoms (defoliation, discoloration, necroses) and signs (galleries, fungal fruiting bodies, spores,). Apart this, other biotic and abiotic damaging factors were recorded. In each locality, symptomatic leaves and branches (i. e. having necrotic lesions) were cut and individually packed into plastic bags. In the laboratory, from three to five 5 mm length segments were cut from each shoot after bark removing. Wood samples were used for pure culture and direct sequencing of fungi. Prior to this, each segment was surface sterilised as follows: 1 min in 96% ethanol, then 5 min in 4% NaClO and 30 s in 96% ethanol (Bakys et al. 2013). Identification of *H. fraxineus* was based on morphological and molecular methods (Gross et al. 2014, Kowalski et al. 2015). Growth of fungal species other than *H. fraxineus* has been recorded, yet those fungi were not further identified. For doubtful isolates absence or presence *H. fraxineus* were confirmed using molecular methods.

Culturing: Collected samples were sterilised and plated on Hagem media supplemented with antibiotics and frozen or fresh ash leaves. Plates were incubated at room temperature and checked daily for fungal outgrowth. All outgrowing fungi were transferred to new Petri dishes and identified by colony morphology and morphological characteristics of its *C. fraxinea* stage (phialophores, spores) and ITS rRNA sequencing (Gross et al. 2014).

Direct sequencing: collected samples were freeze-dried at −60 °C for 2 days. Genomic DNA was isolated using CTAB method. Isolated DNAs were purified using JETquick DNA Clean-Up System. Concentration of genomic DNA was determined using a spectrophotometer. DNA in individual samples were diluted to 1-10ng/µl and amplified by PCR using species specific primers (Gross et al. 2014). The PCR products were analysed using agarose gels.

Pathogenicity tests
One hundred and twenty nine strains of *H. fraxineus* from 10 monitoring plots were paired, resulting in 10 total groups. To investigate the *H. fraxineus* on common ash, inoculation experiments were conducted on 8-9-year-old *F. excelsior* seedlings in forest. In the May 2013, eighty 8-9-year-old trees of common ash were inoculated with selected isolates (8 trees for each isolate). Twenty plants were inoculated with sterile MEA as negative controls. Autoclaved wood pieces of common ash of approximately 1.0x0.2x0.2 cm size were placed on Hagem agar with *H. fraxineus* strain and after colonization with mycelium were used as the inoculum. Inoculations were made by cutting out a bark flap (4 x 8 mm) with a sterile scalpel, placing pre-colonised wood pieces on the exposed sapwood surface and then sealed with Parafilm™ sealing tape, as previously described (Kirisits et al. 2010, Kowalski, Bilanski et al. 2015). The wounds were made on stem, about 0.8-1 m above the root collar. Scalpel and forceps were sterilized using 70% ethanol and open flame before and between every inoculation.

For control, twenty young trees were inoculated with sterile pieces of wood in the same size as the pre-inoculated pieces, using the same inoculation procedure. Observations of plants condition were conducted at weekly intervals for 3 months. After this period, all plants were harvested and the bark was removed around the inoculation site. The length of the necrotic lesion were measured for an estimate of lesion surface area (mm^2). In addition, small pieces of wood tissue 1 cm above of the necrotic stain were cut and plated on Hagem agar medium to re-isolate the fungi. In total, 200 wood pieces were used to re-isolations of fungi. Plates were stored for 3weeks at 22 °C to checked for the presence of the inoculated fungi (Hauptman et al. 2016).

Statistical analyses
The differences in crown damage among plots of different sites were estimated using One-Way Analysis of Variance (ANOVA) with a confidence level of 95%. Chi-square analyses were used to assess the significance of differences between presence of the symptoms of ash dieback and tree condition. The data of inoculation test were analysed using analysis of variance (ANOVA). All calculations were done using statistical software package MinitabTM (Minitab Inc., State College, USA) and PAST: Paleontological Statistics Software Package for Education and Data Analysis (Hammer et al. 2011).

Results

In Ukraine, first observations of ash dieback were made in 2010, mainly on young trees (Davydenko et al. 2013). From 2010 to 2015 ash dieback spread in Ukraine in several areas and damage levels increased dramatically in some stands while other stands looks symptomless, particularly in the East and South regions. Overall, the typical symptoms of ash dieback widespread in all investigated regions but with different extents. Such symptoms were also revealed in urban areas and shelter belts, especially on saplings.

Crown damage, ash trees condition and severity of ash dieback evaluation
To evaluate the damage level of ash trees caused by *H. fraxineus*, monitoring of *F. exelsior* in 6 regions was done during 2011-2015. Among all localities, 69% of the investigated trees have worsened between 2011 and 2015. Only for 5% of all ash trees has increased in their classes while the 32% did not show significant difference in their health condition. Average, the index of ash condition has risen gradually from1.9 to 2.8 (Table 2) on all plots in the period from 2011 to 2015. Up to 2015, the worsening of ash trees condition was significantly higher ($p<0.05$) in Zhytomyr and Kyiv regions (3.9 and 3.3 respectively) while for rest of regions difference between ash conditions varies slightly (2.3-2.6 scores). Since 2011, when only for 0,15 % of the investigated trees presence of *H. fraxineus* was confirmed by molecular methods, the number of affected trees has increased every year up to 88 % in 2015. Worsening rates correlated better with increasing of ash dieback symptoms and pest damage (correlation coefficient: 0.84 - 0.9) than of the climatic conditions for previous period (correlation coefficient 0.23 - 0.43). In 2011, 81,5% of all trees did not have any symptoms of ash dieback regardless of their condition whereas the number of looking-healthy ash trees had fallen to 12% in 2015. The data of disease intensity (Fig 1) showed that about a three fourth of investigated trees remained healthy or showed only weak symptoms of ash dieback (score of weak ash dieback symptoms 0–2) in 2015. Interestingly, that until 2013 the proportion of symptomless or weak

symptomatic trees (score 0-2) was significantly higher (97-100%, $p<0.05$) than in 2015 when it decreased to 74.5% ($p<0.05$).

Table 2 The class of ash trees condition and intensity of ash dieback at the monitoring plots in different regions of Ukraine in 20011-2015.

	Class of ash condition					
Year	Kharkiv	Poltava	Sumy	Kyiv	Zhytomir	Luhansk
2011	1.9	1.9	1.4	2.1	2	2.3
2012	2.1	1.9	1.4	2.1	2	2.5
2013	2.1	2.0	1.9	2.5	2.6	2.5
2014	2.3	2.2	2	2.8	3.0	2.7
2015	2.4	2.2	2.3	3.3	3.9	2.6
	Intensity of ash dieback, score					
	Kharkiv	Poltava	Sumy	Kyiv	Zhytomir	Luhansk
2011	0.1	0	0.3	0.9	0	0.1
2012	0.3	0.1	0.4	1.1	1.1	0.3
2013	0.5	0.2	0.7	1.5	1.9	0.7
2014	1.3	1.3	1.2	1.8	3.0	1.1
2015	1.6	1.5	1.5	2.3	3.8	1.2
Average precipitation level [mm] and temperature 2011-2015						
Precipitation	578	577	588	598	625	557
T° C	7,8	8,1	7	8	7,6	8

For the first time, mortality of ash trees on monitoring plots was observed in 2015 for three regions (Sumy, Poltava and Zhytomyr) and reached 11% of all inspected trees. From the 200 trees sampled, only 24 ones or 12% were symptomless of ash dieback in 2015. No correlation between disease severity and number of healthy trees (correlation index 0.12) was revealed. These disease-free ash trees without damaged by insects or other pathogens, probably, trend to be higher resistance to ash dieback. The one location (Zhytomyr) revealed significantly higher percentage dying or dead trees with score four and five (70%, $p<0.05$) while 62-76.5% ash trees on the other five monitoring plots demonstrated weak symptoms (score 1-2). Even among the dying and dead trees, almost 30% showed no ash dieback infestation.

According to analysed data from regional Forestry Services, the main cause of mortality and following clear-cuts of ash trees in six regions in 2015 were climatic factors (flooding, drought, wind) and root rot diseases including ash dieback coinciding with root rot infection caused by *Armillaria* sp. (Table 3). However, in Kyiv region, the highest rate of mortality (42%) of ash trees caused by bacterial cancer while the peak of insect damage (14%) was observed in Luhansk.

According to our data of causes of ash mortality in monitoring plots, there were significant differences in survey of causes of mortality between study sites in 2015 (Table 3). For 33% of dead trees from study plots *H. fraxineus* coinciding with *Armillaria* sp. was identified whereas only *H. fraxineus* was detected for 24% dead ones. The presence of *Armillaria* sp was verified in the bark samples which have rhizomorphs or typical *Armillaria* - mycelium. The confirmation of *H. fraxineus* was done using cultural isolation and molecular analyses.

Our results show that the collar rot prevalence was observed for the five study sites except Luhansk, where these diseases were not found. Overall, for these five regions (except Luhansk) 84.4 % of the inspected dying and dead trees (4 and 5 classes of condition) showed signs of fungal infections among them 60% root collars had typical *Armillaria* mycelia. Necroses without visible signs of *Armillaria* spp. were observed on 24.4% inspected trees. Isolations from collected samples revealed *H. fraxineus* as a causal agent. The proportion of root collars without any signs of *H. fraxineus* and *Armillaria* spp. (environmental factors in Table 3) was far lower (15.6%). A total of 33.3% of the investigated root collars of the dying and dead trees demonstrated both types of necroses (ash dieback and rhizomorphs or mycelia of *Armillaria* spp).

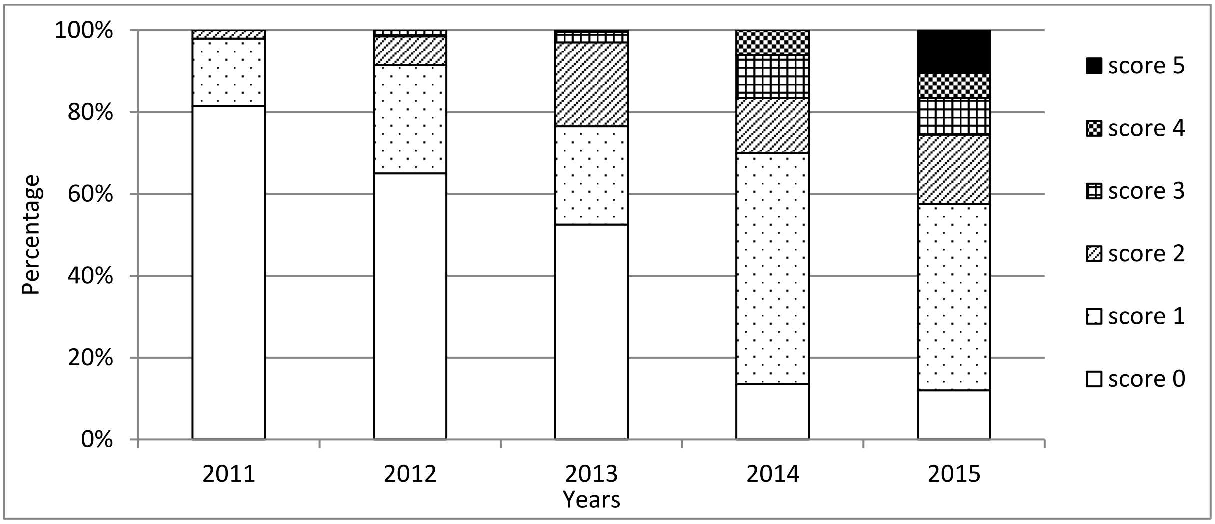


Figure 1 The proportion of ash dieback intensity scores in period from 2011 to 2015 for study sites.

Table 3 Distribution of mortality of ash stands area by the main causes (according to Forest Service data) and inspected ash trees mortality at the monitoring plots in 2015.

	Environmental factors	Root rot (Armillaria)	Bacterial cancer	Ash dieback	Ash dieback and Armillaria	Insects (sawflies, xylophagous etc)
Kharkiv	10.1	27.8	10.1	23.6	27.4	1
Sumy	10.9	33.8	0.2	18	36.7	0.4
Poltava	7	25.6	0.4	27.2	38.8	1.1
Kyiv	23.8	26	42.2	0.4	7.6	0.1
Zhytomyr	19.4	24	2.2	12.3	37.6	4.5
Luhansk	61.9	24.1	0	0	0	14
Inspected ash trees*	15.6	26.7	0	24.4	33.3	0

* Observations of dying and dead trees on all 10 monitoring plots in 2015

Pathogenicity tests

Typical necrotic lesions were a common response to inoculation ash trees with the ten isolates of *H. fraxineus* and no significant difference between treatments was recorded in three months after inoculation. In total, inoculation with *H. fraxineus* resulted in necrotic lesions of different sizes. All strains caused dark-brown submerged lesions in the inoculated ash seedlings. All isolates induced on the inoculated ash trees lesion length of 168-230 mm (173±16 mm), significantly different from the control, p <0.001).The lesions were generally extended in both proximal and distal directions from the wounded area. But lesion lengths were not significantly different by Fishers test ($p \leq 0.05$) following ANNOVA ($n = 8$). Furthermore, no significant differences between wood and bark necroses lengths ($p < 0.01$) were observed.The control treatment gave one very small dark area around inoculation place. At the time of pathogenicity tests both mortality and wilting of branches were observed throughout this experiment in the 8-9-year-old seedlings with all strains. Dead and weakened branches showed typical symptoms of ash dieback such as extending necrosis of stem, wood discoloration and wilt of leaves above inoculation place. No control trees inoculated with sterile agar media had died. We isolated *H. fraxineus* from 78 of 80 inoculated young trees, resulting in a re-isolation success rate of 98%. Branches inoculated with sterile MEA showed no typical ash dieback necroses, and no *H. fraxineus* were re-isolated from control trees.

Discussion

Our results demonstrate that majority of all ash trees in inspected regions showed regularity of gradual increase of ash dieback symptoms and decrease of trees sanitary condition. Our study indicates significant differences in severity of ash dieback in various regions of Ukraine. Therefore, ash dieback has extended across the entire country since 2010. According our personal data and reports from regional Forestry Services, the incidence and mortality of ash trees are the most common for young trees, but it seems to be also spread for all ages. It appears that ash dieback along with other pest and pathogens plays the crucial role in the massive of ash decline in Ukraine as it was previously confirmed in other studies (Cleary et al. 2014, Gross et al. 2014a, Gross et al. 2014b).

Significant differences in the severity of ash dieback among sites could be a reflection of the climate conditions, as drought and low humidity, and arising from them low number of fruit bodies and slow spread of infection into forest. Severe crown damage in such dry regions as Luhansk and Kharkiv could be caused of damage by ash sawlflies or other pests. Moreover, representatives of many fungal pathogenic species were recorded previously in Ukraine (Davydenko et al. 2013) as well as in the previously described studies (Kowalski 2012, Bakys et al. 2013), among them pathogenic fungi (*Alternaria alternata, Aureobasidium pullulans, Hystographium fraxini* etc) which could strengthen ash decline (Przybyl 2002, Kowalski and Łukomska 2005). Therefore, there is a possibility that pathogenicity of certain fungi or crown damage by pests could be triggered by environmental factors.

Up to date, the symptoms of dieback were evenly distributed throughout all examined stands but in different extent. The results of studies in different plantations in Europe indicate differences in resistance to ash dieback between individuals of *F. excelsior* (Cleary et al. 2014, Hauptman et al. 2016, Nguyen et al. 2016). Therefore, significant difference of ash dieback intensity between individual trees in present study could be genetically determined in resistance of *F.excelsior* genotypes to *H.fraxineus* as previously was described (Hauptman et al. 2016). On other hand, we cannot rule out the possibility of synergistic action of several fungi in causing the dieback. The association between *H. fraxineus* and other pathogens in the context of ash decline initiation was not showed well in this study, but this hypothesis can be tested in our further investigation. Furthermore, observed high fungal diversity in declining crowns of ash (Davydenko et al. 2013) along with the most common bacterial diseases caused *Pseudomonas syringae* pv.savastanoi could also led to synergistic effect increasing ash decline intensity (Goychuk and Kulbanska 2014).

Sanitary condition of ash stands in Ukraine has become worse since 2006, when area of drying stands has exceeded 3.4 thousand ha (Davydenko and Meshkova 2014). While multiple causes for dieback on the same tree had been observed as early as 2006, any occurrence of *H. fraxineus* was not demonstrated until 2010 when *H. fraxineus* was found in eastern Ukraine. We could suggest that *H. fraxineus* could appear early in western Ukraine which borders Poland where the disease was reported first in 1992. Probably, necroses caused *H. fraxineus* may be confused with root rot infection, *Armillaria* sp., or bacterial cancer which could develop with *H. fraxineus* simultaneously.

Our results indicate that besides *H. fraxineus*, both *Armillaria* sp. and *P. syringae* pv.savastanoi play an important role as secondary pathogens in ash decline as previously was reported (Skovsgaard et al. 2010, Bakys et al. 2013, Enderle et al. 2015, Hauptman et al. 2016). The third of the investigated dying and dead ash trees revealed that *H. fraxineus* and *Armillaria* spp. present in the same necrosis, although the prevalence of typical mycelia or rhizomorphs of *Armillaria* spp were observed significantly higher than necroses infected with *H. fraxineus*. However, it is possible that most of these trees were initially infected by *H. fraxineus* and *Armillaria* spp. seems to be secondary pathogens as previously was described in numerous studies (Enderle et al. 2015, Hauptman et al. 2016, Nguyen et al. 2016).

Present results of pathogenicity tests indicate that necrotic lesions were formed from all inoculations on ash trees. The all lesions were significantly larger than the control and the pathogen had colonized stem inner bark and spread to the wood around inoculation place. No significant differences between the tested isolates were observed. All necroses represents non-significant different in size and similar symptoms which were morphologically identical to nature infected lesions. The pathogenicity of 10 isolates of *H. fraxineus* were confirmed in our tests that shows their potential to cause damage to *F. excelsior* shoots. Therefore, all the type isolates from necroses exhibited the same pathogenicity level.

Also, our results suggested that the severity of the ash dieback seems to be higher on sites with high air humidity, precipitation and low summer temperatures. However, ash sawfly damage was observed in some dry and hot areas abundantly as an important harmful factor in early spring 2014 that led to far weakening of ash trees.

Conclusions

Dry and hot climate in the east and south parts of Ukraine is probably not as favourable for the wide spread disease as climate of northern part of Europe, where massive ash dieback is observed everywhere. However, we presume that the disease will also in Ukraine significantly reduce ash trees. Moreover, a high level of the *F. exelsior* root collars infected by either *Armillaria* sp., bacterial cancer and *H. fraxineus* raise new questions concerning how these infections contribute ash dieback and which pathogen primarily initiate ash decline in Ukraine.

Acknowledgements

This study was financially supported by the Swedish Institute (SI) and also funded by the EU RTD Framework Programme COST project FP 1103 granting a Short-Term Scientific Mission (STSM) to Dr. Kateryna Davydenko.

References

Bakys, R., R. Vasaitis and J. P. Skovsgaard. 2013. Patterns and severity of crown dieback in young even-aged stands of European ash (*Fraxinus excelsior* L.) in relation to stand density, bud flushing phenotype, and season. *Plant Protection Science.* 49(3): 120-126.

Bakys, R.; Vasaitis, R.; Barklund, P.; Thomsen, I. M.; Stenlid, J., 2009. Occurrence and pathogenicity of fungi in necrotic and non-symtomatic shoots of declining common ash (*Fraxinus excelsior*) in Sweden. *Eur. J. For. Res*. 128, 51–60.

Cleary, M. R., P. F. Andersson, A. Broberg, M. Elfstrand, G. Daniel and J. Stenlid. 2014. Genotypes of *Fraxinus excelsior* with different susceptibility to the ash dieback pathogen Hymenoscyphus pseudoalbidus and their response to the phytotoxin viridiol–A metabolomic and microscopic study. *Phytochemistry*, *102*, 115-125.

Davydenko, K., R. Vasaitis, J. Stenlid and A. Menkis. 2013. Fungi in foliage and shoots of *Fraxinus excelsior* in eastern Ukraine: a first report on *Hymenoscyphus pseudoalbidus*. *Forest Pathology.* 43(6): 462-467.

Davydenko, K. and V. Meshkova. 2014. European ash (*Fraxinus excelsior*) dieback – situation in Europe and Ukraine. *Forestry and landscape gardening*. (5).

Enderle, R., A. Nakou, K. Thomas and B. Metzler. 2015. Susceptibility of autochthonous German *Fraxinus excelsior* clones to *Hymenoscyphus pseudoalbidus* is genetically determined. *Annals of Forest Science.* 72(2): 183-193.

Goychuk, A. and I. Kulbanska. 2014. Etiology of common Ash diseases in Podolia, Ukraine. *Scientific Journal of NUBiP of Ukraine. Series: Forestry and ornamental plants* (198).

Gross, A., O. Holdenrieder, M. Pautasso, V. Queloz and T. N. Sieber. 2014. *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. *Molecular Plant Pathology*. 15(1): 5-21.

Gross, A., T. Hosoya and V. Queloz. 2014. Population structure of the invasive forest pathogen *Hymenoscyphus pseudoalbidus*. *Molecular Ecology.* 23(12): 2943-2960.

Gross, A., T. Hosoya, Y.-J. Zhao and H.-O. Baral. 2015. *Hymenoscyphus linearis* sp. *nov*: another close relative of the ash dieback pathogen *H. fraxineus*. *Mycological Progress.* 14(4): 1-15.

Gross, A., P. L. Zaffarano, A. Duo and C. R. Grunig. 2012. Reproductive mode and life cycle of the ash dieback pathogen *Hymenoscyphus pseudoalbidus*. *Fungal Genetics and Biology*. 49 (12), 977-986.

Gul'chak, V.P. 2012. Державний облік лісів України – підсумки та прогнози [State assessment of the forests of Ukraine – summary and prospects]. *Лісовий і мисливський журнал*. 2: 6–8. (in Ukrainian)

Hammer, O.; Harper, D. A. T.; Ryan, P. D. 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica.* 4, 1-9

Hauptman, T., N. Ogris, M. Groot, B. Piškur and D. Jurc. 2016. Individual resistance of *Fraxinus angustifolia* clones to ash dieback. *Forest Pathology*. DOI:10.1111/efp.12253.

Husson, C.; Scala, B.; Cael, O.; Frey, P.; Feau, N.; Ioos, R.; Marcais, B. 2011. *Chalara fraxinea* is an invasive pathogen in France. *Eur. J. Plant Pathol*. 130, 311–324

Kirisits, T., Matlakova, S. Mottinger-Kroupa, E. Halmschlager and F. Lakatos. 2010. *Chalara fraxinea* associated with dieback of narrow-leafed ash (*Fraxinus angustifolia*). *Plant Pathology.* 59(2): 411-411.

Kirisits, T. and K. Schwanda. 2015. First definite report of natural infection of *Fraxinus ornus* by *Hymenoscyphus fraxineus*. *Forest Pathology.* 45(5): 430-432.

Kowalski, T. 2006. *Chalara fraxinea* sp *nov* associated with dieback of ash (*Fraxinus excelsior*) in Poland. *Forest Pathology.* 36(4): 264-270.

Kowalski, T. 2012. Ash dieback – taxonomical aspects of the disease causal agent. *Sylwan.* 156(4): 262-269.

Kowalski, T., Łukomska, A. 2005. Studies on Fraxinus excelsior L. dieback in Włoszczowa Forest Unit stands. Acta Agrobot. 59, 429–440.

Kowalski, T., P. Bilanski and O. Holdenrieder. 2015. Virulence of *Hymenoscyphus albidus* and *H. fraxineus* on *Fraxinus excelsior* and *F. pennsylvanica*. *Plos One.* 10(10)..

Kowalski, T. and O. Holdenrieder. 2009. The teleomorph of *Chalara fraxinea*, the causal agent of ash dieback. *Forest Pathology.* 39(5): 304-308.

Matsiakh, I.P. and Kramarets V.O. 2014. Всихання ясена звичайного (Fraxinus excelsior L.) на заході України. [Declining of Common Ash (*Fraxinus excelsior* L.) in Western Ukraine]. *Науковий вісник НЛТУ України*:24.7, 67–74. (in Ukrainian)

Mead, R.; Curnow, R. N. 1983. Statistical methods in agriculture and experimental biology. London, Chapman & Hall.

Metzler, B., R. Enderle, M. Karopka, K. Topfner and E. Aldinger. 2012. Development of Ash dieback in a provenance trial on different sites in southern Germany. *Allgemeine Forst Und Jagdzeitung.* 183(7-8): 168-180.

Nguyen, D. T., M. R. Cleary, R. Enderle, A. Berlin and J. Stenlid. 2016. Analyses of the ash dieback pathogen, *Hymenoscyphus fraxineus*, suggest role of tree species diversity on colonization and population structure differentiation. *Forest Pathology*. 46(1): 82-84.

Pautasso, M., G. Aas, V. Queloz and O. Holdenrieder. 2013. European ash (*Fraxinus excelsior*) dieback – A conservation biology challenge. *Biological Conservation*. 158: 37-49.

Przybył, K. 2002. Fungi associated with necrotic apical parts of *Fraxinus excelsior* shoots. *For. Pathol*. 32, 387–394

Sanitary rules in the forests of Ukraine. 1995. Ministry of Forest management of Ukraine.19 p. (in Ukrainian)

Skovsgaard, J. P., I. M. Thomsen, I. M. Skovgaard and T. Martinussen. 2010. Associations among symptoms of dieback in even-aged stands of ash (Fraxinus excelsior L.). *Forest Pathology* 40(1): 7-18.

Queloz, V.; Grünig, C. R.; Berndt, R.; Kowalski, T.; Sieber, T. N.; Holdenrieder, O., 2011: Cryptic speciation in *Hymenoscyphus albidus*. For. Pathol. 41, 133–142.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 228 – 237.

The ash resource and the response to ash dieback in Great Britain

J. CLARK[1], J. WEBBER*[2]

[1] *Earth Trust, Little Wittenham, Abingdon, Oxfordshire, OX14 4QZ,* [2] *Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK*
** Corresponding author: joan.webber@forestry.gsi.gov.uk*

Abstract
In Great Britain, *Fraxinus excelsior* is important both environmentally and as a timber species with an estimated 126 million trees in woodlands and a further 27-60 million in non-woodland environments. The species is more common in Britain than elsewhere in much of continental Europe and forms a major component of several woodland types. It has also been at the centre of the most advanced breeding programme of any broadleaved tree in Britain, and is the only species with tested material available under Forest Reproductive Material Regulations. Inevitably therefore, the impacts of the ash dieback pathogen, *Hymenoscyphus fraxineus*, are likely to be far reaching for both forest economics and ecosystem function. Up until 2012 the ecology of ash was relatively understudied, but the confirmed arrival of *H. fraxineus* in Britain in that year stimulated funding for a study of the role of ash in woodlands. It also opened up new avenues of research to study the genomes of both pathogen and host and discover genetic markers in ash for tolerance to this novel pathogen. Here we report on the existing resource for *F. excelsior* in Britain and summarise the multiple areas of new research focussed around ash dieback, including the infection biology, population genetics, pathogen spread modelling and efforts at disease mitigation. As a first stage in breeding disease tolerant ash, the genome of a low heterozygosity *F. excelsior* individual has been sequenced and, in collaboration with Danish scientists, markers for tolerance identified in a tolerant tree (Clone 35) from Denmark. There have also been early steps in propagating and screening a wide range of *Fraxinus* species and selection of tolerant *F. excelsior* genotypes for a new breeding programme.

Introduction

Of the three *Fraxinus* species found in Europe only the common ash, *Fraxinus excelsior*, is native to Britain (Wallander 2008) where it is one of the most common and familiar broadleaved species. It is the foundation of many valued ecosystems, an important element in the British landscape, and produces valuable timber on relatively short rotations (Kerr and Evans 1993). The species has a broad distribution throughout Europe where it can occur in widely different climates of both maritime and continental character (Pliûra and Heuertz 2003). It can also be present in both the early and the mature stages of woodland succession (Peterken 1993; Pliûra and Heuertz 2003).

Fraxinus excelsior is considered an ecologically flexible species and grows naturally on many different site types throughout Great Britain except in the extreme north-west of Scotland. Wardle (1961) reports that the whole of lowland Britain falls within the range of temperature and rainfall tolerances for *F. excelsior*, with a combination of exposure and unsuitable soil probably responsible for its altitudinal limits. However, it is considered to be nutritionally demanding, growing best on deep, fertile well-drained soils where it can reach a yield class of 10 to 12 (Evans 1984). It rarely forms pure stands of any great extent, occurring more commonly as a component of mixed broadleaved woodland (Joyce 1998). Under the National Vegetation Classification (NVC) for Great Britain, ash is the dominant species of woodland types W8 (*Fraxinus excelsior – Acer campestre – Mercurialis perennis*) and W9 (*Fraxinus excelsior – Sorbus aucuparia – Mercurialis perennis*), is locally common in W7 (*Alnus glutinosa – Fraxinus excelsior – Lysimachia nemorum*) and W12 (*Fagus sylvatica – Mercurialis perennis*) and forms a component of several other woodland communities (Rodwell 1991). Drought tolerance and frost sensitivity also make *F. excelsior* a species likely to be favoured in the short term by climate change (Broadmeadow *et al.* 2005; Scherrer *et al.* 2011). The National Forest Inventory (NFI) for Great Britain surveys woodlands greater than 0.5 ha in area and trees with a diameter at breast height (dbh) greater than 4 cm (Forestry Commission 2013). In 2013, the NFI estimated there were 1.3 million hectares of broadleaves, of which 142 thousand hectares are ash corresponding to 11 % of all broadleaves and 5 % of all species (Figure 1). This is approximately 126 million trees. Furthermore, an estimated 4.2 billion seedlings and saplings were accounted for, of which about 30 % are ash. The greatest concentrations of ash are found in southern England, with a broad band running through the English midlands, but it also occurs at lower densities in Wales and eastern England, being less prevalent in the north of England and Scotland.

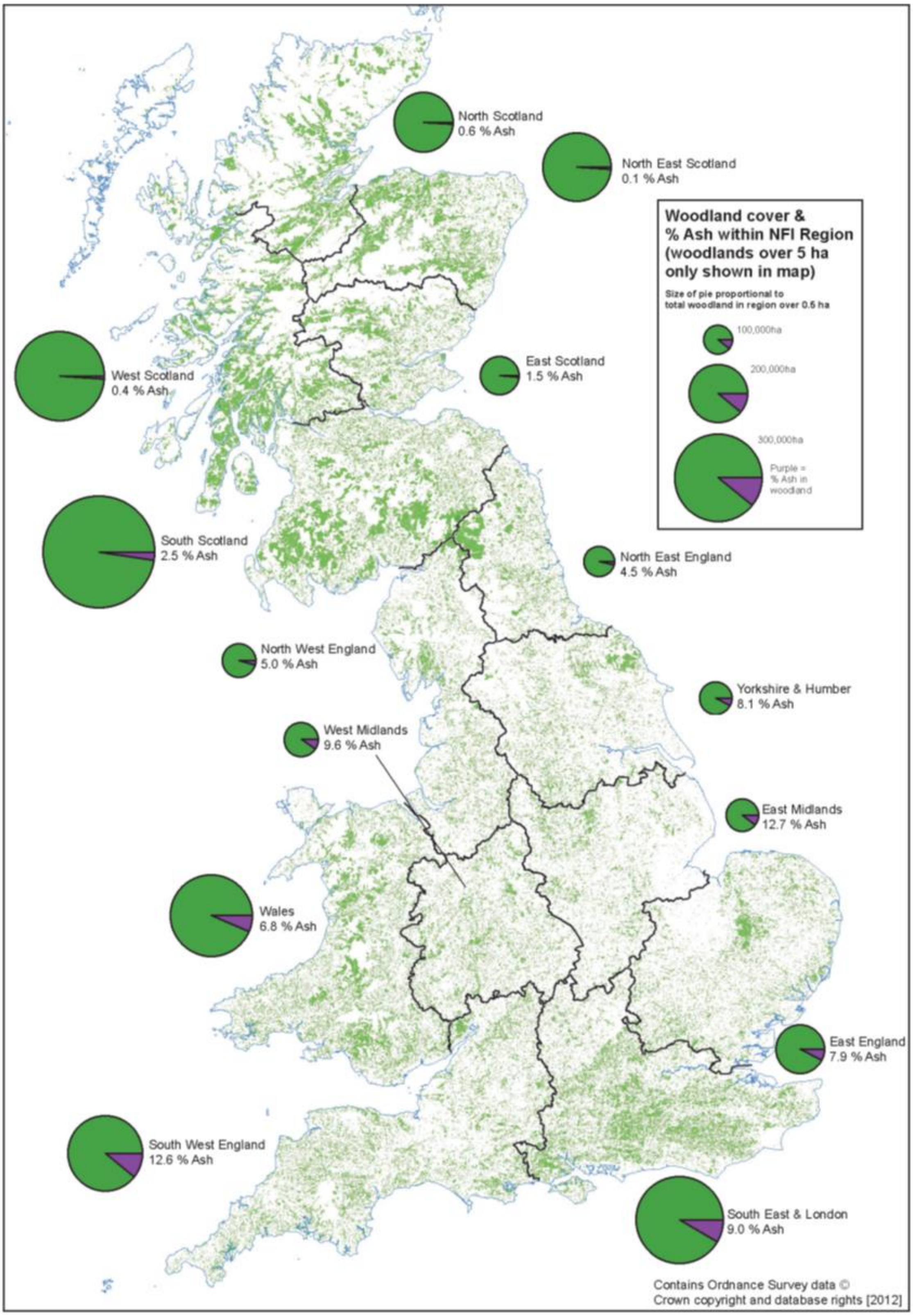


Figure 1 Total woodland cover in Great Britain with proportion of ash by National Forest Inventory region. Forestry Commission 2013. Reproduced from the National Forest Inventory Report (Forestry Commission 2013). Size of pie proportional to total woodland in region: smallest represent forest area up to 100 000 ha, medium up to 200 000 ha, large up to 300 000 ha. Proportion of ash in each of the areas shown in violet.

Ash is also common in non-woodland environments. In 2014, the Tree Council carried out a survey of ash in non-woodland situations on behalf of the Department of the Environment, Food and Rural Affairs (Defra) and estimated there were a further 27-60 million ash trees outside woodland areas with a dbh of more than 4 cm (Defra 2015). This includes 20 million as standards in ash dominated hedgerows (averaging one ash tree every 5 m); 3.6 – 4.0 million ash trees in urban situations; 17 – 34 million in woodlands of less than 0.5ha, and 4 - 4.4 million along the corridors of road and rail networks. In addition to this, the same survey estimated there were a further 400 million smaller ash trees in the form of seedlings and saplings (Defra 2015).

With ash playing such a dominant role in much of Great Britain, and with public fears of a repetition of Dutch elm disease, the UK government has been quick to resource new research into ash dieback following findings of the ash dieback pathogen *Hymenoscyphus fraxineus* in the country. Initially this focussed on providing an understanding of the likely rate of disease spread but with longer term aims of disease mitigation and re-calibration of the already established breeding programme of *F. excelsior.* The research has included an evaluation of the ecological role of ash and its associated species (Mitchell *et al.* 2014a, b, c) with ongoing studies into the genomics of the causal pathogen and host *Fraxinus*, a search for markers indicative of disease tolerance and mass screening of trees for tolerance with which to establish a new breeding programme for ash. As previous work on ash had built up a collection of material from across its natural range of around 40,000 individuals this is also a resource for the current and future research aimed at the creation of resilient ash populations.

Genetic structure of the ash resource in Great Britain

Several studies investigating patterns of genetic diversity within ash have compared variation within and among populations in Europe (Heuertz *et al.* 2004a 2004b; Fraxigen 2005; Sutherland *et al.* 2010 and the RAP project (Realising Ashes Potential, online at www.teagasc.ie/advisory/forestry/rap/) and looked at patterns of local adaptation (FRAXIGEN 2005; Clark 2013).

Heuertz *et al* (2004a) found high levels of genetic diversity with low differentiation between populations; populations from central and western Europe essentially formed a single deme. Work on the genetic variation within British populations by FRAXIGEN (2005), Sutherland *et al.* (2010) and Clark (2013) revealed that the majority of study populations originate from refugia in the Iberian Peninsula (Heuertz *et al* 2004b). Sutherland *et al* (2010) also reported rare and localized individuals identified from the Baltic region as well as three previously unreported haplotypes. Allelic richness differed between sites, decreasing from the east to the west (Sutherland *et al.* 2010) and in the north compared to the south (Heuertz *et al* 2004a). The RAP project also investigated gene diversity, gene flow patterns and hybridisation in ash (between *F. excelsior* and *F. angustifolia*) mostly using microsatellite as genetic markers. These studies indicate that ash in Britain is more genetically diverse than in continental Europe and as a consequence could potentially yield more highly tolerant individuals in response to ash dieback.

Provenance trials

Prior to the arrival of ash dieback disease there has been a focussed breeding programme of *F. excelsior* for improved timber characteristics (form and vigour) in Great Britain. Provenance trials were initially established by Forest Research between 1993 and 1996 on six sites in England and Wales to investigate adaptive variation (Cundall *et al.* 2003). These trials investigated performance of 22 provenances from mostly central and western Europe, but included some from Romania, Yugoslavia, and two from the Czech Republic. The more eastern provenances grew well, but were mal adapted to the maritime climate of Great Britain and suffered from frost damage after early budburst in spring (Cundall *et al.* 2003; Clark 2013) resulting in a high degree of forking. Further trials were established across Europe by the RAP programme in 2003 comprising 45 populations across a range from Ireland to Russia, with 39 provenances represented in the British trial.

Great Britain is divided in to four regions of provenance and 24 native seed zones for all broadleaved species, largely based on climatic and geological variation, with elevation (above and below 300m) a further consideration. In 2007, Forest Research established three trials with two populations from each of 21 of the native seed zones (ash being extremely scare or absent in the three most northerly seed zones). Most recently in 2009, a series of reciprocal transplant experiments along a 2000 km latitudinal transect from Inverness in the north of Scotland to the Pyrenees in the south of France was also established to investigate patterns of local adaptation (Clark 2013). However, early results have not shown evidence of local adaptation; instead some populations have grown well at all sites and the local population never performed best.

Arrival of the disease in Britain

First findings of ash dieback disease

In October 2012, after the first identification of *H. fraxineus* in a nursery (March 2012) and then in a recent landscape planting (May 2012), a small number of affected ash trees were found at sites in the wider natural environment in England, including established woodland in Norfolk and Suffolk. As a result, a comprehensive survey of Britain was conducted based on a grid of 10 x 10 km grid squares. Each 10 km square where ash was known to be present was visited and surveyed for ash dieback disease at three locations. Between October and November 2012, 2500 sites were visited of which 184 (about 7%) were subsequently confirmed as infected. Confirmation of *H. fraxineus* infection at affected sites utilised PCR-based diagnosis using the EPPO protocol (EPPO 2013) and did not just rely on presence of symptoms. The data revealed that the disease was present at a number of infected recently planted sites (ie where young trees had been planted in the preceding 3-5 years and which were probably infected prior to establishment) as well as wider environment sites where large trees in woodlands or hedgerows were affected (Freer-Smith and Webber 2015). Continuing surveillance between 2012-2014 was extensive; it was also both proactive, with areas around the original sites being revisited, and reactive by tracing all sites recently planted with *F. excelsior* or following up of disease reports from forestry professionals and the public. Figure 2 (a, b and c) show the disease distribution of both the recently planted and wider environment sites on completion of the systematic survey in early November 2012, at the end of December 2014 and June 2016, respectively. The figure also illustrates the rapid development of the ash dieback epidemic in Britain, such that by June 2016, 28% of the 10 km squares across Britain with ash were confirmed to have one or more infections of *H. fraxineus*.

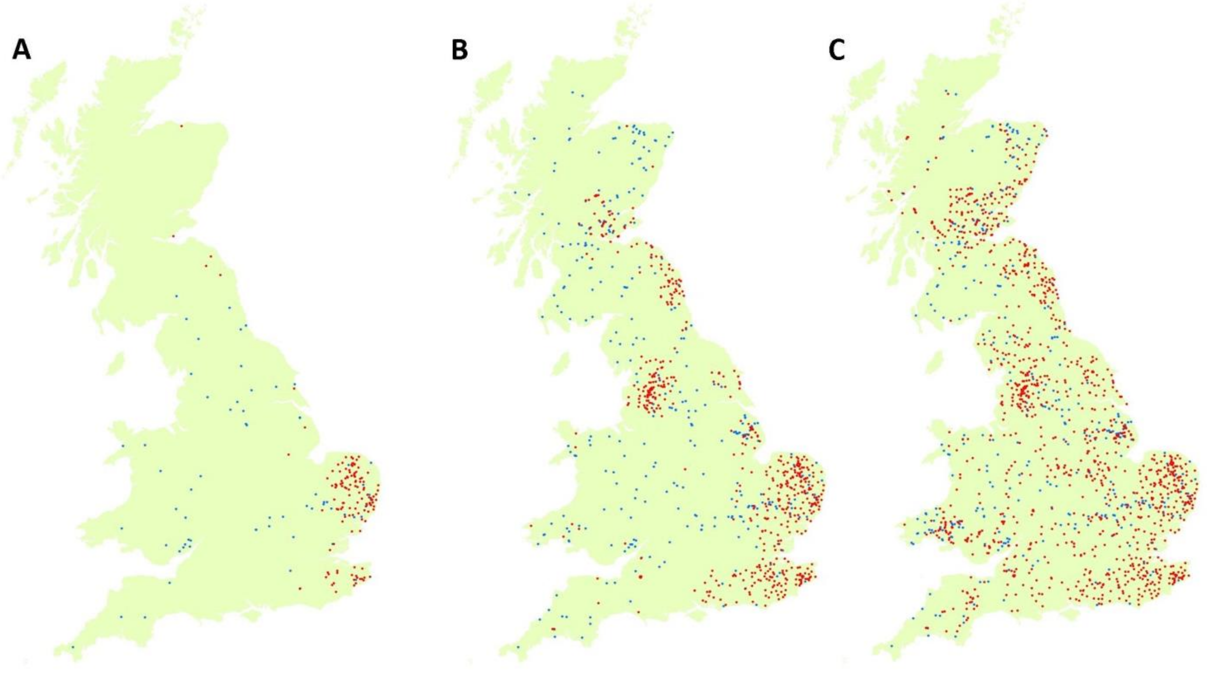


Figure 2 Number of confirmed infections of *Fraxinus excelsior* (European ash) with *Hymenoscyphus fraxineus* since November 2012. Two categories of infected sites are shown: those affecting young trees planted in the preceding 3-5 years in November 2012 (blue dots) and wider environmental sites where large trees in stands or hedgerows are infected (red dots). Map **(A)** shows findings up to November 2012; map **(B)** findings up to December 2014; map **(C)** findings up to June 2016 - © *Crown copyright and database right 2015. Ordnance Survey 100021242. © Crown Copyright 2015 – Land & Property Service No. 130036.*

Apart from formal surveillance, citizen science was harnessed to locate trees affected by ash dieback. In October 2012 soon after the first disease reports Britain, the ‘Adapt Low Carbon Group’ at the University of East Anglia launched AshTag, a smartphone app to enable the public to report trees they suspected of being infected with ash dieback. By submitting photographic evidence via the AshTag app, geo-tagging software provided information on the location of infected trees so that researchers and authorities could follow-up reports and build a picture of where the disease was present. Additionally, aluminium tags could be purchased to mark trees thereby enabling others to relocate trees easily for any follow-up visit. Between the launch in October and December 2012, over 12,000 people installed the app on their smartphones and around 1,000 geo-located photo reports were submitted and authenticated as consistent with ash dieback symptoms. AshTag quickly established that the disease focus was in south east England, particularly East Anglia, although disease reports covered a much wider area including 200 from Scotland.

Regulatory response

Hymenoscyphus fraxineus (or its anamorph *Chalara fraxinea*) has never been listed as a regulated organism in the EC Plant Health Directive which would require action to prevent spread. However, in response to the findings in the UK, emergency legislation at the national level was put in place at the end of 2012 in an attempt to limit any further introductions of *H. fraxineus* by essentially banning all imports of *Fraxinus* plants and seed from regions where the pathogen was known to be present (Anon 2012). This measure was based on evaluations of formal Pest Risk Analyses (Webber and Hendry 2012; Sansford 2013) that infected plants were the most likely entry pathway for *H. fraxineus*, whilst acknowledging that ash dieback was already present in at least some parts of the UK. In addition, during the early stages of the epidemic at least 50,000 ash trees on infected sites were destroyed in an attempt to prevent the disease spread, although the action was applied almost exclusively to recently planted stock.

Disease modelling

Although it was concluded that ash dieback outbreaks at recently established sites were due to use of asymptomatic but infected ash stock, the possibility of disease establishment as a result of spore inoculum wind-blown from continental Europe was also considered. Indeed, the concentration of wider environment infected sites along the eastern seaboard of England, mainly in Kent and East Anglia and sporadically in Lincolnshire and East Yorkshire northwards to Northumberland and Scotland in the early stages of the epidemic (Sansford 2013; Freer-Smith and Webber 2015) suggested that these could have been initiated from long-distance spore dispersal events from other parts of Europe.

To test this hypothesis, a model was developed by University of Cambridge, based on the UK Meterological Office's 'Numerical Atmospheric-dispersion Modelling Environment'. This model strongly supported the likelihood of airborne incursion from Europe (M. Castle and R. Cox unpublished 2013). It concluded that between 2008 and 2011 there were at least 100 days on which environmental conditions including wind direction, rainfall and humidity, could have carried *H. fraxineus* spore masses from infected ash in mainland Europe across the English Channel or North Sea and caused infection of ash predominantly in east and south-east England (Downing 2012; Wentworth 2012). Epidemiological modelling of the likely progression of the ash dieback epidemic in Britain also indicated that the pathogen was likely to continue to spread in Great Britain although with potential for significant regional variation with areas in the south east, east and south west most likely to be affected in the early stages of the epidemic.

Research response – the host

Existing work on ash has resulted in a large, genetically diverse collection of material from across its natural range and for all categories of Forest Reproductive Material, amounting to approximately 40,000 individuals. This diverse resource has enabled several research bodies to tackle the impacts of ash dieback in Britain and will continue to contribute significantly to future research. Under an urgency grant from the Natural Environment Research Council in 2013, researchers from Queen Mary University, London sequenced the genome of a British *F. excelsior* tree of low heterozygosity produced by controlled self-pollination through the FRAXIGEN project. The results are published on the British Ash Genome Project website www.ashgenome.org.

When the Nornex consortium (http://nornex.org/) started work on ash dieback in 2013, one of the aims was to identify genetic resistance in ash through genome sequencing and mapping (Downie 2016). Sequencing focussed on the genome of the ash-dieback-tolerant tree (Clone 35), identified as part of the Danish breeding programme (Kjear *et al.* 2011; McKinney *et al.* 2011) and which has been shown to be highly tolerant of *H. fraxineus.* Additional references for ash genome analysis also included the British *F. excelsior* tree of low heterozygosity (www.ashgenome.org). Genome annotation of *F. excelsior* was then carried out to differing degrees depending on the assemblies available and differential expression analysis revealed distinct expression patterns between resistant/tolerant and susceptible clones of *F. excelsior* to *H. fraxineus*. The next stage of research included the use of associative transcriptomics to identify markers associated with disease susceptibility in *F. excelsior* exploiting *H. fraxineus*-infected tissue from two different clones with extreme phenotypes (one highly susceptible and one of low susceptibility - namely Clone 35). A total of three genetic markers were identified, two GEM and one cSNP markers, associated with tolerance within Clone 35 but not present in the susceptible clone (Harper *et al.* 2016). The same markers were also found to occur in *Fraxinus* species that are considered to have very low susceptibility to ash dieback: *F. americana*, *F. ornus* and *F. mandshurica*. The markers were then tested as predictors of tolerance, using tissue from a panel of about 200 trees from Denmark with known levels of susceptibility and a further group of trees from Britain exposed to the disease for several years which had developed different levels of crown dieback, and successfully identified individuals with low levels of susceptibility (ie those with few crown symptoms of ash dieback). Building on this work, it is planned that ash populations from the Europe wide RAP trial, and individuals from a British wide clonal seed orchard of phenotypically superior parents will be assessed using these markers to predict tolerant individuals.

Two Defra funded projects are also looking directly for ‘field’ tolerance within ash population through mass screening of field resources. In 2012, when the UK ban on movement of ash came into force, forest nurseries were preparing for the field season with tens of thousands of ash seedlings ready for planting, most of which were then unsaleable due to the combined impact of the disease and the ban. The first project (Rapid screening for *Chalara* resistance using ash trees currently in commercial nurseries: TH0132) utilised some of this material and Forest Research established 14 field trials across East Anglia where *H. fraxineus* was already prevalent in the wider environment. Forty eight hectares were planted in the spring of 2013, with 155,000 seedlings from 15 seed sources (ten British native seed zones, two Irish, two from continental Europe and one improved British of qualified status). These plots have been monitored each year since planting. In 2014, all sites had some infected plants but overall survival was 91 %. However, by the end of 2015 in total over 50 % of trees were infected across all sites (Lee 2016). Ultimately the intention is to screen any individual trees that survive continuing exposure to the disease in anticipation that they are likely to prove tolerant to *H. fraxineus.*

The second Defra funded project (Selection and breeding of common ash for resistance to *Chalara fraxinea*: TH0133) is screening the existing resource already described (see Genetic structure of the ash resource). Called the Living Ash Project (http://livingashproject.org.uk), and using an estimate of 1% of screened trees showing a good degree of tolerance (>10 % crown dieback) to ash dieback (Kjear *et al.* 2011), the project aims to identify 400 putatively tolerance individuals from which to commence a new breeding programme. Additionally, trees in the wider environment, but of source identified status, are being screened using citizen science via AshTag. Since the use of AshTag to identify diseased trees in 2012, it has now been re-launched so that tagged trees can be monitored over time to follow disease progress and identify trees which potentially appear to be less susceptible to ash dieback because of their survival in highly infected areas; a 10,000 tags have been given away to encourage this initiative. In addition, a new series of ash progeny trials have been planted in spring 2016 using the tested seed from the original breeding programme. Paternity analysis is being undertaken, to enable both parents to be identified within any tolerant individuals arising from the trials. Finally, tissue culture and cryopreservation techniques are being developed to enable the rapid bulking up of tolerant genotypes. Any tree being put forward as putatively tolerant will be screened using the markers developed through the Nornex consortium.

Research response – the pathogen

In the early stages of the epidemic in Britain, findings in relation to the pathogen were consistent with those discovered elsewhere. Both idiomorphs or sexual mating types (MAT 1-1 and MAT 1-2) were found in populations of *H. fraxineus* regardless of whether these came from infected nursery stock or wider environment outbreaks (Open Ash Dieback hub http://oadb.tsl.ac.uk). Further work also showed that the pathogen has a somatic self–nonself recognition or vegetative compatibility ‘vc’ system that is readily expressed in culture. Moreover, in highly localised isolate samples almost all isolates are incompatible indicating high *vic* gene diversity and therefore an out-breeding life cycle (Brasier and Webber 2013). High levels of incompatibility between individuals also suggest that it might be difficult to deploy damaging fungal viruses against the pathogen as a disease control method, because viruses usually spread more readily in a fungal population with high levels of clonality which allow mycelium of different individuals to fuse and facilitate the transfer of deleterious agents such as viruses.

In depth genetic analyses by the Nornex consortium have also generated a reference genome for the ash dieback pathogen *H. fraxineus* using an isolate from Norfolk as the reference strain; transcriptome data for *H. fraxineus* have then been used to identify genes expressed during the fungal lifecycle (Downie 2016). When diversity in *H. fraxineus* was compared using historical accessions of *H. fraxineus* from across Europe, recently obtained isolates from Britain and isolates from Japan (Downie 2016), the genome sequence data were consistent with an extreme population genetic bottle-neck indicating just a few individuals of the pathogen have initiated the epidemic in Europe. Therefore, although *H. fraxineus* has high levels of outcrossing there is limited variation in the population structure, even at the epidemic front.

At the time that the Nornex project started there were no reproducible *in vitro* tests of pathogenicity in ash seedlings using either conidia (asexual spores) or ascospores (sexual spores) as a means of following infection by *H. fraxineus*. However, pursuing the idea that conidia could be involved, and through the use of confocal microscopy imaging, these asexual spores were shown to germinate on ash leaves *in vitro* and on artificial substrates, albeit with low efficiency. When similar conidial spore suspensions were applied to leaves of six week old ash seedlings symptoms were expressed which included browning of leaf veins and tips, progressing to leaf drop (Fones *et al.* 2016). Intriguingly this suggests that infection of ash leaves may not just caused by ascospores as previously thought, but may also be effected through asexual spores which had previously been though to act only as spermatia (Gross *et al.* 2012, 2014).

There have also been other research initiatives. Another major Defra funded project (Mitigation of the impacts of ash dieback in the UK – TH0119) which is still ongoing includes studies to identify the durations of spore release in UK outbreaks and measure the volumes of spores released into the air to provide core data for modelling of pathogen spread. Additionally, experiments in the laboratory and natural outbreaks are examining the susceptibility of ash species commonly grown in the UK and aim to determine how seasonal factors and inoculum levels affect infection rates. The work is also evaluating the safe disposal of infected tree material such as fallen leaves and twigs through processes such as composting and how readily this can eradicate *H. fraxineus*. In October 2014, the first details of the laboratory and field work on testing the efficacy of currently available fungicides against ash dieback were published (Anon 2014). The most effective compounds were either triazoles or carboxamides which were found to reduce both foliar symptoms and stem lesions induced on young trees in field trials. Although it is recognised that chemical treatments are unlikely to be economic when applied to trees in woodlands they could have a useful role in protecting specific high value trees, although repeated treatments would probably be required. Any successful treatments would also need to be integrated into good practice guidelines for management of ash dieback.

Management options and conclusions

Britain's broadleaved woodlands are dominated by relatively few species, which can limit management options when deciding what to do in ash dieback infected woodlands. While Scotland still has a thriving timber industry mainly based on conifers, many woodlands, particularly in England, are managed for conservation and amenity objectives, with public access often playing a key role. Early outbreaks of ash dieback were originally contained, with new plantations being removed and burnt on site. However, as the disease spread to the wider environment, management objectives for older woodlands have aimed to slow the spread and lessen the impacts of the disease. Now, the principle aims of management are to:

- Maintain the values and benefits associated with ash woodlands and iconic trees;
- Secure an economic return where timber production is an important objective;
- Reduce the presence and rate of spread of *Chalara* ash dieback;
- Maintain as much genetic diversity in ash trees as possible with the aim of ensuring the presence of ash in the long term;
- Minimise impacts on associated species and wider biodiversity.

In some regions especially where *F. excelsior* forms pure stands, the disease has also been viewed as an opportunity to increase species diversity and build resilience into ecosystems such as the ash woods of the Peak District in northern England.

Across Britain, disease levels are still patchy with only 28% of the 10 km squares across the country with one or more infections of *H. fraxineus* in 2016 Ash dieback has been found in all counties in England to varying extent, but some ash dominated areas of Wales and the south west and southern Scotland are still relatively disease free. However, the south east of England, where the disease was first encountered, is heavily infected with many young ash stands suffering very high mortality. Although this suggests very rapid disease progression based on the first reports of *H. fraxineus* in Britain in 2012, a more complex picture is also emerging. A recent study has provided evidence that some ash stock apparently infected with *H. fraxineus* was planted in England as early as the 1990s, with affected trees dying of the disease in the mid-2000s (Wylder et al. 2016). This places *H. fraxineus* in England much earlier than previously thought, and suggests there can be a lag phase of more than a decade before outbreaks start to have an impact on the landscape.

As the disease progresses different strategies are required to allow land managers time to intervene and adapt woodland management prescriptions in the expectation of high losses to come. Maintaining the structure and value of any woodland should be the primary aim of management, while avoiding accelerating any decline in condition. In heavily infected areas, it is now advised that woodlands should be thinned as usual to promote canopy development while focusing on retaining species other than ash. It can be difficult to ensure that the ecological role realised by ash trees is maintained as no one species encompasses all the functions of ash. Those trees showing the most extreme dieback symptoms should be favoured for removal and the lop and top burnt on site where possible. Although some landowners are felling ash to realise the economic benefits before ash dieback has established, the most common end use for ash in the current market is for firewood. This means that the rush to fell ash before it becomes infected is lessened, and tolerant trees are therefore likely to remain, potentially allowing for natural regeneration with some degree of tolerance.

Where infection is absent or at low levels, a ‘business as usual approach’ has been recommended in high forest situations to maintain tree vigour and retain a full canopy. Trees to be selected for thinning which show symptoms of ash dieback are most easily assessed in the summer when symptoms are most visible. If only small numbers of recently planted or naturally regenerated ash show symptoms, it may be beneficial to remove the entire planting to slow the wider spread of disease and allowing time to establish new trees of other species. No one species will fulfil the ecological niche of ash. However, work by Mitchell *et al.* (2014 a, b, c) has attempted to quantify the role of ash. Out of 955 species that utilise ash trees in some way, 45 species (largely invertebrates) are reported to be obligate ash users, with a further 62 species being highly associated, rarely using other species. Planting beech (*Fagus sylvatica*) and oak (*Quercus*) species (where appropriate) will facilitate a large number of these highly associated species, as will sycamore (*Acer pseudoplatanus*) and hazel (*Corylus avellana*). However, the ecosystem function of ash, in terms of both levels of light within a woodland and rate of decay of leaf litter, is better served by supplementing ash with small leaved lime (*Tilia cordata*) and common alder (*Alnus glutinosa*).

The UK has historically imported a large proportion of its planting stock from Europe, even when the seed may have been of UK origin. With the confirmation of the ash dieback pathogen in a nursery in 2012, the risks associated with disease introductions via imported nursery stock were highlighted. This has led to much wider questioning within the industry about seed sourcing, the history of material as it is grown and moves through the supply chain, and been the launchpad of initiatives such as the Grown in Britain Campaign (http://www.growninbritain.org). Government guidance now routinely recommends using UK grown planting stock, and UK origin, but suggests diversifying in terms of species and provenances choice to include near continental material, whilst still promoting use of British grown stock. Looking forward, the foundations are already in place to predict some of the factors associated with disease tolerance, to identify trees with low disease susceptibility and thereby rebuild the ash population in Britain in the future.

Acknowledgements

This paper draws on research by many organizations within the UK, funding for which was supported by Research Councils BBSRC and NERC, the Forestry Commission and the Department for Environment, Food and Rural Affairs (Defra). The cooperation that led to this review paper was supported by funding from COST Action FP1103 FRAXBACK and the Forestry Commission.

References

Anon. 2012. The Plant Health (Forestry) (Amendment) Order 2012. Statutory Instrument 2012, No. 2707. In force 29 October 2012. 6pp.

Anon. 2013. Results from preliminary screening of approved fungicides for efficacy against *Hymenoscyphus fraxineus* (*Chalara fraxinea*), the cause of ash dieback file:///C:/Users/OWNER/Downloads/12430_Shortreportonresultsfromfungicideefficacytestingforchalarav2.pdf

Brasier, C. and Webber, J., Vegetative incompatibility in the ash dieback pathogen *Hymenoscyphus pseudoalbidus* and its ecological implications. *Fungal Ecology* 6(6) 501-512. http://dx.doi.org/10.1016/j.funeco.2013.09.006

Broadmeadow, M.J.S., Ray, D. and Samuel, C.J.A. 2005. Climate change and the future for broadleaved tree species in Britain. *Forestry* 78: 145-161.

British Ash Genome Project 2013. http://www.ashgenome.org (accessed on 5 February 2016).

Clark, J.R. 2013. Adaptation of ash (*Fraxinus excelsior* L.) to climate change. Ph.D. Thesis, Bangor University, UK.

Cundall, E.P., Cahalan, C.M. and Connolly, T. 2003. Early results of ash (*Fraxinus excelsior* L.) provenance trials at sites in England and Wales. *Forestry* 76: 385-399.

Downie, A. 2016. Nornex Final Report: An open consortium (Nornex) for molecular understanding of ash dieback disease. http://oadb.tsl.ac.uk/wp-content/uploads/2016/04/Nornex_Final_Report_April_2016.pdf (accessed on 15 May 2016).

Defra 2015. Chalara in Non-Woodland Situations. http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=19770&FromSearch=Y&Publisher=1&SearchText=ash dieback &SortString=ProjectCode&SortOrder=Asc&Paging=10#Description (accessed on 15th May 2016).

Downing, E. 2014 *House of Commons Library - Ash dieback disease: Chalara fraxinea.* http://www.parliament.uk/business/publications/research/briefing-papers/SN06498/ash-dieback-disease-chalara-fraxinea (accessed on 7 January 2016).

EPPO. 2013 PM 7/117 (1) *Hymenoscyphus pseudoalbidus*. *EPPO Bull*. 43: 449–461.

Evans J. 1984. *Silviculture of Broadleaved Woodland*. Forestry Commission Bulletin 62. HMSO London.

Fones, H.N., Mardon, C. and Gurr, S.J. 2016. A role for the asexual spores in infection of *Fraxinus excelsior* by the ash-dieback fungus *Hymenoscyphus fraxineus*. Submitted to journal.

Freer-Smith, P.H. and Webber, J.F. 2015. Tree pests and diseases: the threat to biodiversity and ecosystem services. *Biodivers. Conserv.* 24 DOI: 10.1007/s10531-015-1019-0.

Forestry Commission, 2013. National Forest Inventory estimates of quantities of broadleaved species in British woodlands, with special focus on ash. HMSO, Edinburgh.

FRAXIGEN, 2005. Ash species in Europe: biological characteristics and practical guidelines for sustainable use. Oxford Forestry institute, University of Oxford, UK. 128 pp.

Gross, A., Zaffarano, P.L., Duo, A. and Grünig, C.R. 2012. Reproductive mode and life cycle of the ash dieback pathogen *Hymenoscyphus pseudoalbidus*. *Fungal Genetics and Biology* 49(12): 977-986.

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V. and Sieber, T.N. 2014. *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. *Molecular Plant Pathology* 15(1): 5-21.

Harper, A.L., McKinney, L.V., Nielsen, L.R., Havlickova, L., Li, Y., Trick, M., Fraser, F., Want, L., Fellgett, A., Sollars, E.S.A., Janacek, S.H., Downie, J.A., Buggs, R.J.A., Kjear., E.D. and Bancroft, I. 2016. Molecular markers for tolerance of European ash (*Fraxinus excelsior*) to dieback disease identified using associative transcriptomics. *Scientific Reports* 6: 1-7.

Heuertz, M., Hausman, J.F., Hardy, O.J., Vendramin, G.G., Frascaria-Lacoste, N. and Vekemans, X. 2004a. Nuclear Microsatellites reveal contrasting patterns of genetic structure between western and southeastern European populations of the common ash (*Fraxinus excelsior* L.). Evolution 58 (5): 976-988.

Heuertz, M., Fineschi, S., Anzidei, M., Pastorelli, R., Salvini, D., Paule, L., Frascaria-Lacoste, N., Hardy, O.J., Veksemans, X. and Vendramin, G.G. 2004b. Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe. *Molecular Ecology* 13: 3437-3452.

Joyce, P.M. 1998. *Growing Broadleaves. Silvicultural Guidelines for Ash, Sycamore, Wild Cherry, Beech and Oak in Ireland.* COFORD, Dublin.

Kerr, G. and Evans, J. 1993. *Growing Broadleaves for Timber*. Forestry Commission Handbook 9. HMSO, London.

Kjear, E.D., McKinney, L.V., Nielsen, L.R., Hansen, L.N. and Hansen, J.K. 2011. Adaptive potential of ash (*Fraxinus excelsior*) populations against the novel emerging pathogen *Hymenoscyphus pseudoalbidus*. *Evolutionary Applications* 5: 219-228.

Lee, S. 2016. Screening ash for Chalara. Ash Newsletter April 2016. Forest Research, Northern Research Station, Edinburgh. 4pp.

McKinney, L.V., Nielsen, L.R., Hansen, J.K. and Kjear E.D. 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (Oleraceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. *Heredity* 106: 788-797.

Mitchell, R.J., Beaton, J.K., Bellamy, P.E., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Gimona, A., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Iason, G.R., Kerr, G., Littlewood, N.A., Newey, S., Potts, J.M., Pozsgai, G., Ray, D., Sim, D.A., Stockan, J.A., Taylor, A.F.S. and Woodward, S. (2014a). Ash dieback in the UK: a review of the ecological and conservation implications and potential management options. *Biological Conservation* 175: 95-109.

Mitchell, R.J., Beaton, J.K., Bellamy, P.E., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Gimona, A., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Iason, G.R., Kerr, G., Littlewood, N.A., Newey, S., Potts J.M., Pozsga,i G., Ray, D., Sim, D.A., Stockan, J.A., Taylor, A.F.S. and Woodward, S. (2014b). *Assessing and addressing the impacts of ash dieback on UK woodlands and trees of conservation importance (Phase 2)*. Natural England Commissioned Reports, Number 151.

Mitchell, R.J., Bailey, S., Beaton, J.K., Bellamy, P.E., Brooker, R.W., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Farren, J., Gimona, A., Goldberg, E., Hall, J., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Hooper, R.J., Howe, L., Iason, G.R., Kerr, G., Littlewood, N.A., Morgan, V., Newey, S., Potts, J.M., Pozsgai, G., Ray, D., Sim, D.A., Stockan, J.A., Taylor, A.F.S. and Woodward, S. 2014c. *The potential ecological impact of ash dieback in the UK.* Joint Nature Conservation Committee Report (JNCC) 483. JNCC Peterborough. 222 pp.

Peterken, G.F. 1993. *Woodland Conservation and Management*. Chapman and Hall, London.

Pliûra, A. and Heuertz, M. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for common ash (*Fraxinus excelsior*). International Plant Genetic Resources Institute, Rome, Italy. 6 pages.

Rodwell, J. 1991 *British Plant Communities. Volume 1, Woodlands and Scrub.* Cambridge, Cambridge University Press.

Sansford C.E. 2013. *Pest Risk Analysis for Hymenoscyphus pseudoalbidus (anamorph Chalara fraxinea) for the UK and the Republic of Ireland.* http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/hymenoscyphusPseudoalbidusPRA.pdf (accessed on 7 January 2016).

Scherrer, D, Bader, M.K.F. and Körner, C. 2011. Drought-sensitivity ranking of deciduous tree species based on thermal imaging of forest canopies. *Agricultural and Forest Meteorology* 151: 1632-1640.

Sutherland, B.G., Belaj, A., Nier, S., Cottrell, J.E., Vaughan. S.P., Hubert, J. and Russell, K. 2010. Molecular biodiversity and population structure in common ash (*Fraxinus excelsior* L.) in Britain: implications for conservation. *Molecular Ecology* 19: 2196-2211.

Wallander E. 2008. Systematics of *Fraxinus* (Oleaceae) and evolution of dioecy. *Plant Systematics and Evolution* 273: 25-49.

Wardle P. 1961. Biological flora of the British Isles *Fraxinus excelsior* L. *Journal of Ecology* 49: 739-751.

Wentworth J. 2012. Transmission of ash dieback. POSTbox, December 2012. Houses of Parliament, Parliamentary Office of Science and Technology. http://www.parliament.uk/documents/post/Ashdieback_POSTbox.pdf (accessed on 7 January 2016).

Webber, J. and Hendry, S. 2012. Rapid assessment of the need for a detailed Pest Risk Analysis for *Chalara fraxinea*. Version 1.2, 9th August 2012, 15pp.

Wylder, B., Biddle, M., King, K., Baden R. and Webber, J. 2016. Mortality dating of *Fraxinus excelsior* to demonstrate the early presence of ash dieback (*Hymenoscyphus fraxineus*) in England. *Forestry* (submitted).

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 238 – 248.

Differences in susceptibility to *Hymenoscyphus fraxineus* (dieback of ash) of selections of Common ash (*Fraxinus excelsior*) in The Netherlands – Report of the observations and results of 2012 and 2015

SVEN M.G. DE VRIES*, J. KOPINGA

Alterra, Wageningen University & Research Centre
**Corresponding author: sven.devries@chello.nl*

Abstract

In 2012 and 2015 assessments have been carried out in the genebank and seed orchards of the State Forest Service in order to evaluate the level of attack of common ash caused by the fungus *Hymenoscyphus fraxineus (Chalara fraxinea).*The aim of the study was to obtain insight in possible genetically determined differences of susceptibility for the disease within the existing clonal selections of ash that are present in The Netherlands at this moment.

A broad variation in leaf infection and twig dieback was observed amongst over 200 clonal selections ranging from 0 to100 on a scale of 0-100. Correlation between the rate of leaf infection and the rate of twig dieback was significant, but not consistent. This could partly be explained by differences in protection mechanisms (so called 'avoidance') of the trees to the disease. In 2015 it turned out that some clones looked healthier compared to 2012, while other clones were more attacked. It looks as if the disease has not yet been stabilised. Since the disease is only just present in The Netherlands since 2010 and a further expansion is to be expected within the coming years, no hard statements can yet be made about the definite level of susceptibility without further tests such as controlled inoculations.

A number of commercial clones already showed an above average level of tolerance to the disease. Pollarded or heavily pruned trees showed a significant higher level of susceptibility to both leaf infection as well as to twig dieback than non pollarded or heavily pruned trees. Also did the female clones in this group of selections show to be significantly more susceptible to both leaf infection as well as to twig dieback than the male clones.

Keywords: *Fraxinus excelsior*, *Hymenoscyphus fraxinues*, *Chalara fraxinea*, Ash dieback ADB, clonal selections, natural infection

1. Introduction

In 2010 and 2011 an increasing amount of announcements of disease attacks in plantations of ash in different parts of the country (particularly in the provinces of Flevoland and Utrecht) were made. These attacks were caused by the fungus *Chalara fraxinea* (asexual), today known as *Hymenoscyphus fraxineus* (sexual). In Western-Europe were attacks of the fungus so far particularly observed on common ash (*Fraxinus excelsior*), which is indigenous in The Netherlands. But also attacks were observed, although to a lesser extent on narrow leaved ash (*Fraxinus angustifolia*), which is less frequently present compared to common ash and which is not native in our country (Raad voor plantenrassen, 2007).

The disease appeared in the beginning most strikingly in plantations were the trees were coppiced, but later on also more and more in commonly managed forest areas. From 2011 and onwards the disease was also found in forest tree nurseries and nurseries for roadside trees.

It is expected that the disease will spread out in The Netherlands just like it has done in Poland, Germany and Scandinavia (Bosschap, 2011; Vereniging voor Natuurmonumenten, 2011), with all the same disastrous consequences to forests, natural areas and the landscape in the countryside.

It is to be expected that the disease will have great impact on both managers of forests and other kind of plantations, but also on producers and traders of reproductive material of ash, both in the nurseries as well as in trade. For managers, nurseries and trade it would be useful to know on short notice whether they should continue growing ash. And if the answer to this question would be positive, the next question would be whether this should be done with the current clones and provenances or maybe start producing reproductive material with a higher genetic level of resistance that is less susceptible to Ash dieback (ADB).

There are indications that not every individual or every provenance of common ash will suffer in the same way to attacks of this disease. For instance research by McKinney *et al.* (2011) showed that in Denmark there exists a broad variation in the reaction of different genotypes to ADB. Also in Sweden susceptibility differs between different individual ash trees (Bakys *et al*. 2009). Furthermore, our own observations in the Seed orchards in Vaartbos and the genebank Roggebotzand, both from the Dutch State Forest Service, but also other plantations in The Netherlands in 2011 showed differences in the symptoms to ADB on individual tree level. This offers positive prospects for the selection of resistant or even more tolerant basic material of ash in The Netherlands.

The objective of the research described in this paper meant to be in the first place an inventory of the behaviour and reactions to ADB of basic material (clones) that already was positively selected and was found to be satisfactory and “well-known” regarding all kind of traits other than ADB.

2. Materials and methods

A first series of assessments was carried out in 2012 in the gene bank Roggebotzand (NB/OL: 52.33.28 / 5.47.44) near Dronten as well as in a replicate thereof in nursery 'Reeshof' (NB/OL: 51.34.35 / 5.00.23) near Tilburg and in the seed orchards Vaartbos -01 and -02 (NB/OL: resp. 52.20.43 / 5.47.44 en 52.30.53 / 5.29.24) close to Zeewolde.

This concerns stands in which a large number of Dutch selections of ash are present as solitaire trees in several replications. This enabled us not only to assess the complete tree crowns of different ash clones, but also to compare more individuals within the same clone with each other. Already in 2011 some indicating field assessments took place in the gene bank Roggebotzand near Dronten as well as in the seed orchards of ash, Vaartbos -01 and -02 near Zeewolde. At that occasion branches from infected ash trees from the gene bank have been sampled for evaluation to identify the cause of the infection. The NVWA (the Dutch authority for food and trade) in Wageningen isolated the fungus and identified it as *Hymenoscyphus fraxineus*. On the basis of this it was decided to start the assessments on the for this disease so typical phenotypic characters: twig dieback and leaf infection, carried out by making use of evaluation systems already described in relevant literature.

The assessments in 2102 have been carried out on several dates during the period between June and October. The results of which have been reported in 2013 (Kopinga & De Vries, 2013). The situation as it was found in week 36 and 38 in 2012 was set as the starting point for evaluation of the situation in 2015. A second series of assessments was carried out in 2015 in Roggebotzand en Vaartbos -01 en -02. Reeshof was already cleared in 2013, at which occasion a few of the trees were replanted in the gene bank Roggebotzand.

Part of the seed orchard Vaartbos -01 was also cleared in the beginning of 2015, but the amount of remaining trees was still found to be enough for a –statistical- evaluation between the clones. The observations in 2015 were limited to a single assessment on 21 September (week 39) 2015. Because some of the trees at that point already had dropped their leaves for different reasons than ADB (‘early autumn’) the observations have been limited to only assess the amount of twig dieback. The amount in which the trees were infected by ADB is given as percentage of infected twigs within the tree crown.

The phenotypical traits that were particularly observed were the presence and the amount of recently died twigs in combination with the scorching of recently flushed leaves; this according to Koltay et al. (2012). In order to be able to compare our work in the near future in connection with most of the other international observations and assessments we introduced the scoring methods of Pliura et al. (2011), McKinney et al. (2011) and Kirisits & Freinschlag (2012) in comparison with our own methods. These methods are all based on a ranking of the estimated percentages of infected or dead twigs within the tree crown. In the systematics of the research reported in this paper we used a ranking system in some cases and after a while we also made use of percentages, all according to whether it concerned older or younger trees we dealt with or if it concerned twig dieback or leaf infection that was the most relevant symptom of the disease.

3. Description of the plantations ‘Roggebotzand’, ‘Reeshof’ and ‘Vaartbos’

Roggebotzand and Reeshof are plantations established with pollarded trees derived from a collection of basic material from the seventies of which clonal reproductive material for the production of roadside trees should be delivered. Originally this collection was created and managed by The Forestry Research Institute “De Dorschkamp” in Wageningen with plant material from the Foundation for the Improvement of Trees (Stichting

Verbetering Houtopstanden, SVH). Originally there were kept 4 ramets of each of the clone numbers; after the change of ownership of the location in 2006 the ash trees had to be removed and two ramets of each of the 142 different clones were removed to the gene bank Roggebotzand en two to the nursery Reeshof near Tilburg. Except for few of the trees the plants all survived well. All trees are pollarded every other two years at a height of 1.2 meters above ground level. The advantage of having Reeshof as a replication of the gene bank Roggebotzand (both locations lie115 kilometres apart from each other) makes it possible to compare regional differences in the behaviour of each of the 142 clones. The disadvantage on the other hand is that every clone is only represented by two individuals each, which could be seen as a statistical limitation to any of the observations or assessments. Therefore it can only be allowed to preliminary base conclusions regarding ADB in fully grown adult trees on the observations in these pollarded trees of just 4 individuals per clone. In both plantations five commercially traded and widely used clones along roadsides in The Netherlands act as reference clones (Altena, Atlas, Eureka, Geessink and Westhof's Glorie).

Vaartbos -01 (74 different selected clones) en -02 (38 different selected clones) lie in close proximity of each other (on a distance of 350 meters) and are established with two year old plants in 1990/1991 as seed orchards for the production of forest reproductive material of ash. Of each of the selected clones 6 ramets were planted in the seed orchard lay-out design. Also in both these plantations five commercially traded and widely used clones along roadsides in The Netherlands act as reference clones (Altena, Atlas, Eureka, Geessink and Westhof's Glorie). From each of these clones 12 ramets were planted. The ash trees in these two seed orchards can be accepted as adult trees. At regular intervals a third of the total number of the trees is heavily pruned in order to promote the possibilities of seed harvests. The advantage of this situation was that 6 ramets per clone enabled us to have statistically more sound based conclusions of our observations, while at the same time also the influence of heavily pruning of the trees on the symptoms of ADB could be observed. With exception of three of the commercially traded clones there is no overlap between the two seed orchards Vaartbos -01 and -02. However, some of the clones that are represented in the gene bank are also represented in one of the two seed orchards, as this was also the case for the five commercially traded clones. This enabled us to compare the behaviour of several clones that occurred both as pollarded trees in the gene bank and as adult trees in the seed orchards.

4. General Results

It was remarkable that in 2012 none of all the plantations Roggebotzand, Reeshof and Vaartbos showed newly appeared twig dieback from 2012, not even later in the season. But what did appeared from June-July on were leaf infections that are so characteristic for ADB, that in some cases already within a few weeks lead to wilting of leaves and early leaf fall, sometimes followed by flushing of new leaves.

Gene bank Roggebotzand, 1 October 2012. The different clones are planted in duplicate within the row.

Seed orchard Vaartbos, August 2012.

5. Results gene banks Roggebotzand and Reeshof

In June 2012 there was no evidence for a clear indication of leaf infection in either of the two gene banks, although it was possible to evaluate the amount of twig dieback from 2011. Figure 1 shows the situation of the clones in the gene bank Roggebotzand. Each of the bars shows the average twig dieback of the two ramets for each of the clones in 2011. The position for each of the five commercially traded and widely used clones is indicated by a bold bar.

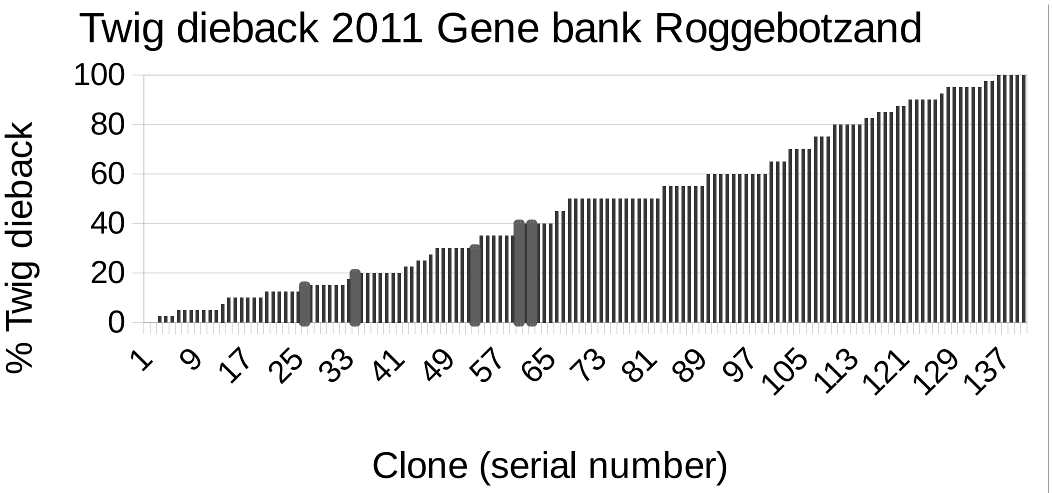


Figure 1 Bar chart of the average twig dieback of the clones in 2011, assessed on the 19th of June 2012 in the gene bank Roggebotzand, arranged by increasing susceptibility. Each bar represents one clone. The position of five commercially traded and widely used clones along roadsides are indicated by bold bars. From left to right: Westhof's Glorie < Altena < Atlas < Eureka < Geessink. Horizontal axis: clone serial number (numbered by increased susceptibility). Vertical axis: estimated percentage of dead twigs in the tree crown.

In the gene bank Reeshof it was decided not to make an inventory on the amount of twig dieback, since the manager of this gene bank had already pruned heavily infected branches, which would give an incomplete picture of any kind of evaluation.

Figure 2 shows the situation regarding the differences in leaf infection in 2012 as an avarage of the two ramets for each of the clones in Roggebotzand, while figure 3 shows the same for Reeshof. Both assessments took place in the beginning of September 2012. Also in these two figures the position for each of the five commercially traded and widely used clones is indicated by a bold bar. Compared to figure 1 there are less clones in Roggebotzand in 2012 than there were in 2011, because some of the clones didn’t flush or died completely and were therefore not taken into account any longer.

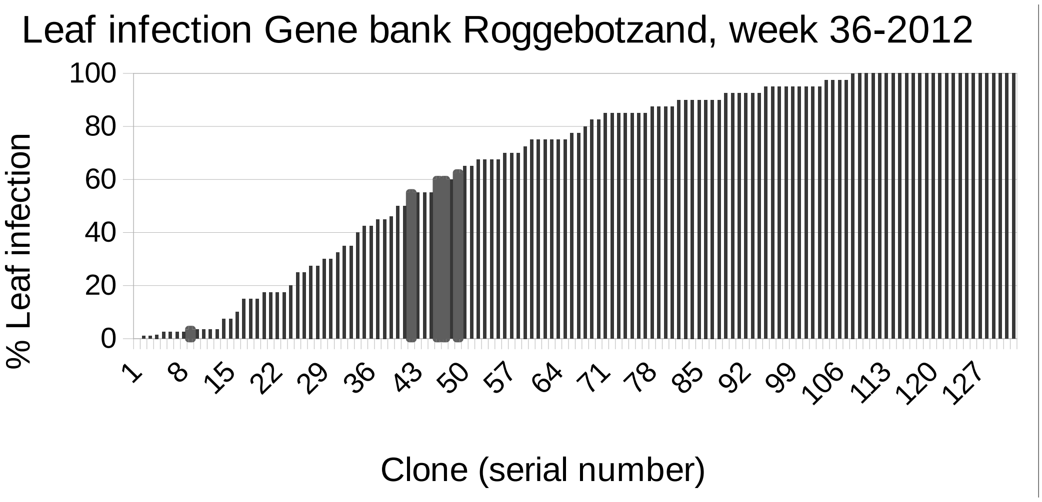


Figure 2 Bar chart of the average percentage of leaf infection of the clones, assessed on 5 September 2012 in the gene bank Roggebotzand, arranged by increased susceptibility. Each bar represents one clone. The position of five commercially traded and widely used clones along roadsides are indicated by bold bars. From left to right: Altena < Eureka < Westhof's Glorie = Geessink < Atlas. Horizontal axis: clone number (numbered by increased susceptibility). Vertical axis: estimated percentage of infected leaves in the tree crown.

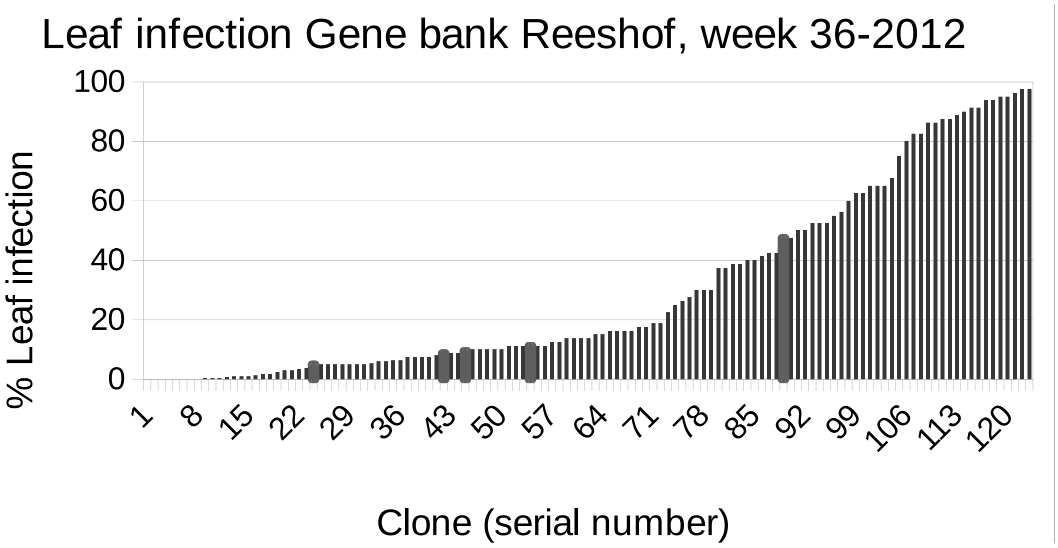


Figure 3 Bar chart of the average percentage of leaf infection of the clones, assessed on 6 September 2012 in the gene bank Reeshof, arranged by increased susceptibility. Each bar represents one clone. The position of four commercially traded and widely used clones along roadsides are indicated by bold bars. From left to right: Atlas < Altena < Eureka < Geessink < Westhof's Glorie. Horizontal axis: clone number (numbered by increased susceptibility). Vertical axis: estimated percentage of infected leaves in the tree crown.

We noticed a striking difference between both assessments, that were executed with just a day difference (5 and 6 September 2012); it showed that the disease in Reeshof developed at a different speed than in Roggebotzand. This was already shown earlier during the growing season when we made our observations. In Roggebotzand the average percentage of leaf infection of all trees at the 14th of August 2012 was 30,5%, which was increased to 64,6% at the 5th of September. In Reeshof the average percentage of leaf infection of all trees at the 20st of August 2012 was 30,6%, but this stayed more or less the same with 29,7% after three weeks on the 6th of September.

Also the position of some of the commercially traded and widely used clones in the figures 2 and 3 turned out to be different. Only Altena and Westhof's Glorie turn out to be more or less the same. The same affect can be seen at some of the other clones. A possible explanation will be given in the 'Discussion' chapter of this paper.

6. Results seed orchards Vaartbos -01 and -02

In June 2012 neither of the two seed orchards in Vaartbos showed any clear signs of leaf infection. However, the amount of twig dieback resulting from 2011 was clearly visible and this was assessed in 2012. Herewith we still made use of our own scoring system in which the trees were classified in a ranking of 0 – 7, from "healthy" to "heavily diseased".

Figure 4 shows the position for each of the clones in both seed orchards together. Every bar shows the average twig dieback of the clones based on at least 4 ramets per clone. The position of the five commercially traded and widely used clones along roadsides are indicated by a red colour.

Figure 5 shows the differences in leaf infection halfway September 2012. The position of the five commercially traded and widely used clones along roadsides are indicated by a red colour.

For many of the clones it turned out that the level of twig dieback differs very much from the level of leaf infection. Even though there is a significant overall correlation between twig dieback and leaf infection (Spearman's rho = 0.68 at p = 0.000), but the somewhat low correlation value gives reason to suspect some amount of variation. This is also shown in figure 6 where among others a rather high level percentage of leaf infection in the group of twig dieback with ranking 0 is due to just a few out layers with high levels of percentages of leaf infection that increase the average rather heavily. This means that there are clones where a high level of leaf infection does not come together with a high level of twig dieback. In figure 6 a rather high dispersion of the percentage of leaf infection is shown, particularly in the lower- middle area of the ranking.

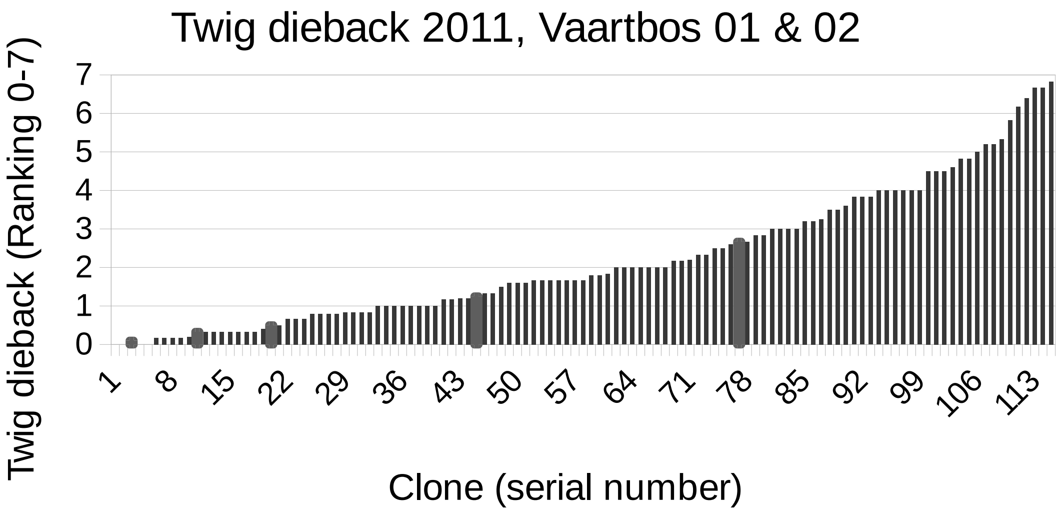


Figure 4 Bar chart of the average twig dieback of the clones in 2011, assessed in June 2012 in the seed orchard Vaartbos -01 & -02, arranged by increased susceptibility. Each bar represents one clone. The position of five commercially traded and widely used clones along roadsides are indicated by bold bars. From left to right: Atlas < Geessink < Westhof's Glorie < Altena < Eureka. Horizontal axis: clone number (numbered by increased susceptibility). Vertical axis: ranking of twig dieback in the tree crown ranging from 0 = healthy (0% dieback) to 7 = very severely affected (95-100 % dieback).

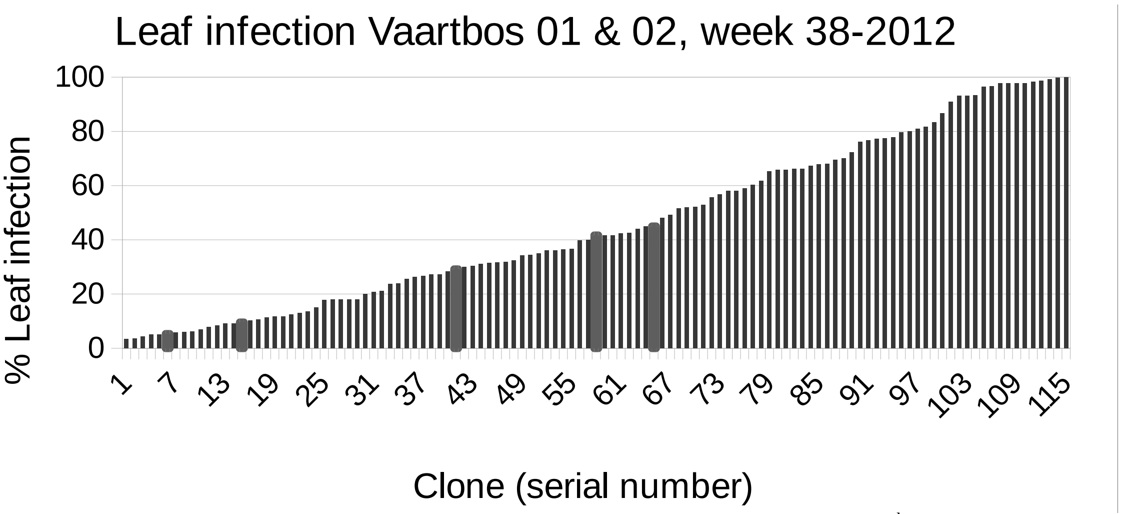
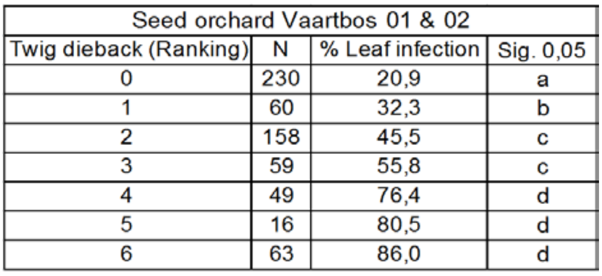


Figure 5 Bar chart of the average leaf infection of the clones in 2012, assessed on the 19th of September 2012 in the seed orchard Vaartbos, arranged by increased susceptibility. Each bar represents one clone. The position of five commercially traded and widely used clones along roadsides are indicated by bold bars. From left to right: Atlas < Altena < Geessink < Westhof's Glorie < Eureka. Horizontal axis: clone number (numbered by increased susceptibility). Vertical axis: estimated percentage of infected leaves in the tree crown.

Table 1 Percentages of average leaf infection for each of the rankings of twig dieback. Sig. 0.05 represents the statistical significance of the means at p=0.05 according the Mann-Whitney test, in which values with the same letter are not statistically different.



Seed orchard Vaartbos 01 & 02			
Twig dieback (Ranking)	N	% Leaf infection	Sig. 0,05
0	230	20,9	a
1	60	32,3	b
2	158	45,5	c
3	59	55,8	c
4	49	76,4	d
5	16	80,5	d
6	63	86,0	d

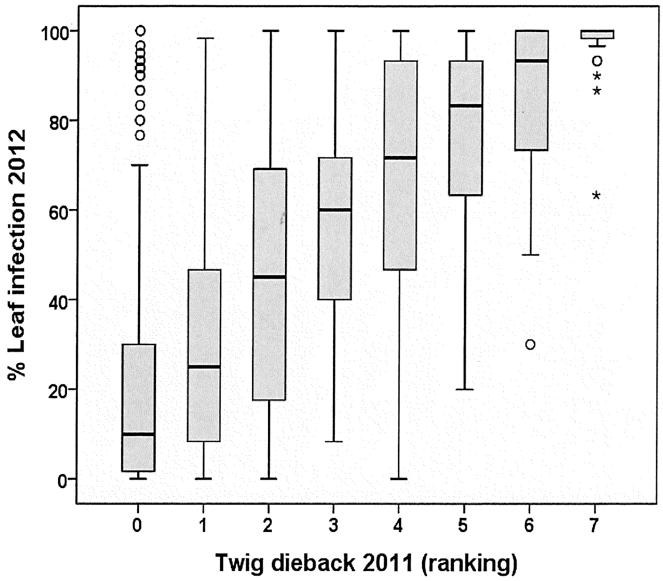


Figure 6 Box plot of the percentages of average leaf infection for each of the rankings of twig dieback presented in Table 1. Horizontal axis: Ranking of twig dieback (0 = healthy; 7 = very severely affected). Vertical axis: estimated percentage of infected leaves in the tree crown.

7. Influence of heavily pruning

From the 661 trees still present in Vaartbos -01 & -02 the crowns from 296 trees were heavily pruned in 2009 due to a more efficient seed harvest. The average ranking of twig dieback and leaf infection in 2011 as well as twig dieback in 2015 is shown in table 2 for both heavily pruned and of non-pruned trees. In both years there was a significant difference between the group "pruned trees" and "non-pruned trees" (Mann-Whitney, p = 0,000). This shows that it makes quite a lot of difference for both twig dieback as well as leaf infection whether the trees are pruned or not.

Table 2 Difference in average ranking of twig dieback (on a scale of 0 to 7) and the average percentage of infected leaves of heavily pruned and of non-pruned trees in 2011 and 2015.

	Seed orchard Vaartbos 01 & 02				
	2011			2015	
Pruned	N	Twig dieback (Ranking)	Leaf infection (%)	N	Twig dieback (%)
No	365	1,9	35,8	257	37,1
Yes	296	2,5	58,0	221	49,4
Total	661	2,2	45,8	478	42,8

8. Influence of gender

All clones descent from trees that are either male, female or both male and female (hermaphrodite) trees. The differences between these three groups regarding twig dieback (2011 and 2015) and leaf infection (2012) of the trees in in Vaartbos -01 & -02 are shown in table 3. The same is given for the gene bank Roggebotzand in table 4.

9. Comparison of observations in 2015 with those in 2012

The correlation between the ranking of twig dieback from 2011 and the percentage of twig dieback in 2015 in Vaartbos -01 & -02 was, despite the fact that the amount of trees was less in 2012 still significant (Spearman's Rho: 0,642 at p = 0,000). The relatively low R-value shows some changes in position which can be observed in figure 7, that shows the situation in both the seed orchards Vaartbos -01 & -02. Some individual trees however have seemed to be recovered, while other trees turned to be even more diseased in the meantime.

Table 3 Difference in average ranking of twig dieback (on a scale of 0 to 7) and the average percentage of infected leaves of female, male and hermaphrodite clones in the seed orchards Vaartbos -01 & -02. Sig. 0.05 represents the statistical significance of the means at p=0.05 according the Mann-Whitney test, in which values with the same letter are not statistically different.

	Seed orchard Vaartbos 01 & 02								
	2011			2012			2015		
Gender	N	Twig dieback (ranking)	Sig 0,05 (M-W)	N	Leaf infection (%)	Sig 0,05 (M-W)	N	Twig dieback (%)	Sig 0,05 (M-W)
Not indicated	212	2.08	b	212	45.2	b	146	37.9	b
Male	115	1.45	a	115	34.6	a	93	26.8	a
Female	334	2.46	b	334	49.9	b	239	52.0	c
Total	661	2.16	n.a.	661	45.8	n.a.	478	42.8	n.a.

Table 4 Difference in average percentage of twig dieback and the average percentage of infected leaves of female, male and hermaphrodite clones in the gene bank Roggebotzand. Sig. 0.05 represents the statistical significance of the means at p=0.05 according the Mann-Whitney test, in which values with the same letter are not statistically different.

		Gene bank Roggebotzand					
		2011		2012		2015	
Gender	N	Twig dieback (%)	Sig 0,05 (M-W)	Leaf infection (%)	Sig 0,05 (M-W)	Twig dieback (%)	Sig 0,05 (M-W)
Not indicated	62	36,3	a	67,5	b	61,6	ab
Male	34	28,8	a	47,9	a	49,4	a
Female	121	38,8	a	64,9	b	63,9	b
Total	217	36,52	n.a.	63,0	n.a.	61,0	n.a.

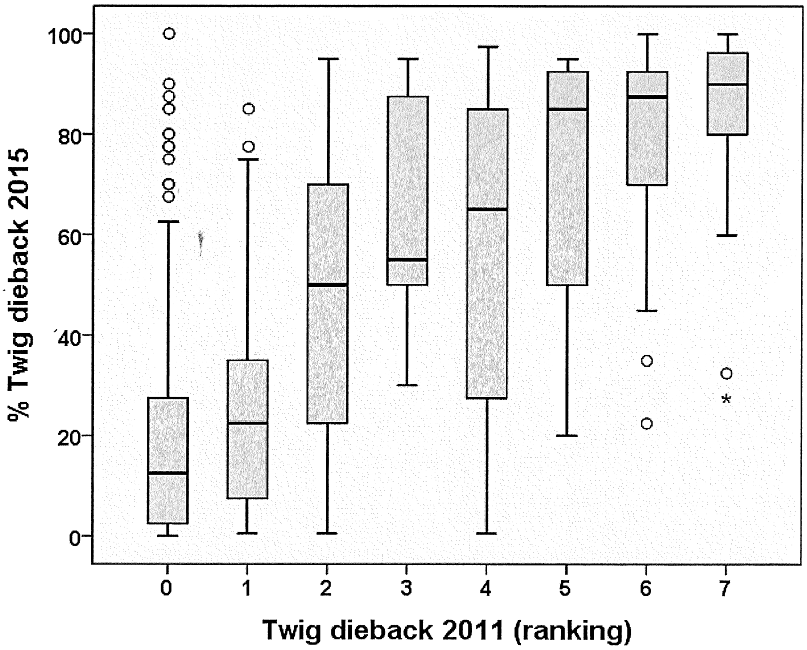


Figure 7 Boxplot of the average percentage of twig dieback in 2015 compared to the average ranking of twig dieback in 2011 (on a scale of 0 to 7).

Many of the clones have stayed in the same position regarding the amount of twig dieback, while other clones have moved their positions more towards either ‘less tolerant’ or ‘more tolerant’. The differences in ranking of the position of the five commercially traded and widely used clones in relation to all the clones is shown in table 5. Only those clones that were still alive in 2015 and that have been observed for ‘pruned’ or ‘non-pruned’ have been taken into account.

Table 5 Ranking (see figure 4) of the position of the five commercially traded and widely used clones along roadsides in the total picture of twig dieback in the seed orchards Vaartbos -01 & -02 in relation to all the clones present in these 2 seed orchards (N=115 in 2015).

	All trees			Non-pollarded			Pollarded		
		2011	2015		2011	2015		2011	2015
Clone (N=115)	N	Ranking	Ranking	N	Ranking	Ranking	N	Ranking	Ranking
Atlas	5	1 - 9	8	3	1 - 15	3	2	1 - 23	12 - 13
Altena	10	44	10	6	17 - 20	4	4	50 - 60	18
Westhof's Glorie	10	18	44	6	16	25	4	32	44
Eureka	11	67 - 83	65	8	79	65	3	42 - 43	57
Geessink	4	11 - 13	68	3	18	77	1	1 - 23	31

The situation in the gene bank Roggebotzand is somewhat different (see table 6). 'Altena' and 'Geessink' turn out to be even a bit more favourable as in 2011. ‘Atlas’ however shows to be less favourable, while 'Westhof's Glorie' and 'Eureka' stay more or less the same.

Table 6 Ranking (see figure 1) of the position of the five commercially traded and widely used clones along roadsides in the total picture of twig dieback in the gene bank Roggebotzand in relation to all the clones present in this genebank (N=118 in 2015).

	Twig dieback	
	2011	2015
Clone (N=217)	Ranking	Ranking
Atlas	61 - 67	86 - 89
Altena	34 - 42	8 - 11
Westhof's Glorie	27 - 32	28 - 84
Eureka	61 - 67	57 - 59
Geessink	68 - 82	30 - 35

10. Discussion

It turned out that regarding both leaf infection and twig dieback in the seed orchards Vaartbos -01 and -02 alone there is a rather high amount of clones (app. 40) that have a low level of susceptibility to ADB in which they do not statistically differ from each other. This includes the cultivars Atlas, Altena, Geessink en Westhof's Glorie. Eureka could in both cases be considered somewhere in the middle between low and high susceptibility. This can be considered very promising in case there should be a wish to increase the amount of (moderately) tolerant clones with clones that have not yet been issued before, but from which there is already quite a lot of technical information gathered regarding behaviour and growth.

In case the disease symptoms between the same genotypes on different locations give similar results there would be a very good basis for further selection of suitable basic material of ash. The advantages of this approach are in short:

- The provenance of the trees is already known and described
- Much of the behaviour of the genotypes in relation to their adaptability to the Dutch circumstances is already known: this enables a quick release as cultivars
- Since this material is planted in many different trials, there are enough replications for statistical sound conclusions
- All the trees in trials and seed orchards differ in age and are therefore ideal for assessments of disease susceptibility at different age categories

In general testing for disease susceptibility of trees from which much traditional trait information is already known is to be preferred above selecting trees in nature on the basis of good results regarding disease tolerance, that subsequently have to be tested for other desirable traits.

There are several indications that the disease has not really settled completely in The Netherlands yet and therefore it can be expected that the intensity of the disease can still increase. Even though the average level of twig dieback in 2015 was higher than in 2011, there can still not be given a general trend, because first of all the assessments in 2015 were carried out only once, secondly could early leaf fall have disturbed the observations and third there have not been made any observations in between 2011 and 2015.

This means that the development of the disease has to be observed for a while longer and besides this has material of promising genotypes regarding resistance to be tested under more conditioned circumstances, like inoculation trials before clones could trustworthy be released as cultivars. The necessity for inoculation trials is supported by the ‘shift’ in the ranking in the amount of twig dieback in the period 2011-2015 of some of the clones, among which were also a few commercial cultivars.

What makes that a clone shows significantly more leaf infection (followed by a quick defoliation) than twig dieback, or the other way around, is not yet fully clear. A deviation like this cannot be explained by the ‘way of observing’. It is true that the estimations of the amount of leaf infection (and defoliation) and twig dieback are seldom zero, because also completely healthy trees sometimes have several dead twigs, but nine clones with a with a leaf infection of 80-100% with trees without any twig dieback at all points towards other causes and connections like differences in mechanisms of resistance. It could for instance be due to the resistance mechanism known as 'avoidance' in which a tree sheds its leaves prematurely in order to avoid the fungus to enter the twigs through affected leafs. This makes it interesting in inoculation trials to inoculate both the petiole as well as the leaf tissue of the twig, or maybe to spray the leaves with a spore suspension in order to better mimic natural circumstances. However, so far twig inoculation seems to be the most ‘harsh’ and discriminating method to test the level of susceptibility.

Also there is no conclusive explanation for the differences in leaf infection between the gene banks Roggebotzand and Reeshof. The vegetation in Reeshof is grass that is kept short and relatively clean, while that of Roggebotzand is of a higher and rougher nature with grasses and herbs with extensive maintenance. It could very well be that solely the infection pressure from fruit bodies on shed leaves that stay under the trees is less in Reeshof than it is in Roggebotzand. But given the rather big regional differences in which the disease has expressed its self so far it could also be other locally connected ecological factors, like the differences in weather and/ or microclimate that were of influence.

Neither is there a conclusive explanation for the average higher levels of infection of heavily pruned trees with relatively much new wood in relation to non-pruned trees. Perhaps the fact that heavily pruned trees stay stressed for a while after the pruning operation, what keeps them more sensitive for secondary infections or weather influences (like frost) in general. But also the fact that fungus grows more easily in tissue of younger wood than in older tissue could play a role. This has so far not been subject to investigation as far as we know.

These findings fit very well with recommendations that so far have been given to administrative and management organisations like the Society for Forest and Nature Owners and the Society for Nature monuments not to prune ash trees too heavily when there is no real need for it. Nevertheless do the differences between Roggebotzand and Vaartbos indicate that perhaps it differs whether it concerns relatively young pollarded trees or heavily pruned older trees. But because of the low amount of replications of the clones present in Roggebotzand there could not be made a sound conclusion based on these comparisons.

What strikes is the difference in susceptibility to ADB between male and female clones. This cannot be due to any subjectivity in the observations (an abundant seed load could influence these) because first of all the gender of the trees was known beforehand and secondly did the trees in the gene bank, apart from just very few, not carry any seeds yet. An explanation for this difference between genders is so far not available.

Now that it is revealed that there exist major differences in infections between vegetative propagated Dutch clonal selections, it becomes interesting to investigate to what account the level of resistance is carried over to the offspring of open pollinated mother trees (halfsibs). Also it seems interesting to observe the average levels of resistance of Dutch provenances that are nowadays recommended in the National Catalogue and that are used in forestry. Especially because the last few years foreign research showed significant differences both between halfsib families as well as between provenances in susceptibility to ADB.

For obvious reasons the question arises to what extend plant material from controlled pollinated crossings could be obtained with an even higher level of resistance. This comparable with the work carried out regarding the Dutch Elm Disease breeding programs from the past 60 to 70 years. The latter could become specifically important when it turns out that despite sophisticated choice of basic material the level of ADB could not be brought back to acceptable proportions, but it could of course also act as an additional solution.

11. Conclusions and recommendations

There exist an enormous variation in the level of infections with Ash Dieback (ADB) between clonal selections in The Netherlands that range from 0 to 100 on a scale from 0-100. This as such justifies the work to create a bigger 'pool' of suitable basic material of commercial cultivars for roadside plantations.

In forestry in general, where seedling populations of ash derived from provenances and seed orchards are used as reproductive material, there is a need for more insight in the levels of resistance of the offspring from this basic material, that in many cases is used already today. This needs to be further investigated.

The results found so far are still premature. This means it is still not clear to what extent the intensity as well as the spread of the disease will increase. The somewhat big differences in the amount of twig dieback shown by some of the clones in 2015 in relation to 2011 demand for some moderation regarding firm conclusions. It deserves recommendation to keep on following the development as well as the amounts of infection for the coming years and to investigate the level of resistance on the basis of test trials under more conditioned circumstances.

Literature

Bakys, R. Vasaitis, P. Barklund, K. Ihrmark & J. Stenlid. 2009. Investigations concerning the role of *Chalara fraxinea* in declining *Fraxinus excelsior*. Plant Pathology 58, 284-292.

Bosschap. 2011: www.bosschap.nl/cms/.../8.../Praktijkadvies%20essentaksterfte.pdf.

Chandelier, A., N. Delhaye & M. Nelson. 2011. First Report of the Ash Dieback Pathogen *Hymenoscyphus pseudoalbidus* (Anamorph *Chalara fraxinea*) on *Fraxinus excelsior* in Belgium. The American Phytopathological Society, APSnet Vol 95, nr 2, 220.1.

Hietala, A.M. & H. Solheim. 2011. *Hymenoscyphus* species associated with European ash. QEPP/EPPO Bulletin 41, 3-6. EPPO Archives. 2010. Workshop on Chalara fraxinea, Oslo, Norway 2010-06-30/07-02.

Kirisits, T. & C. Freinschlag. 2012. Ash dieback caused by *Hymenoscyphus pseudoalbidus* in a seed plantation of *Fraxinus excelsior* in Austria. Journal of Agricultural Extension and Rural Development Vol. 4(9), pp. 184-191.

Koltay, A.; I. Szabo & G. Janik. 2012. *Chalara fraxinea* incidence in Hungarian ash *Fraxinus excelsior*) forests. Journal of Agricultural Extension and Rural Development Vol. 4(9), pp. 236-238.

Kopinga, J. & S.M.G. de Vries. 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*). Report nr. 26. Centrum voor Genetische Bronnen Nederland (CGN), Wageningen, Wageningen UR (University & Research centre). 31 p.

Kowalski, T. 2006. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelsior* in Poland. Forest Pathology 36, 264-270.

Mc Kinney, L.V., L.R. Nielsen, J.K. Hansen & E.D. Kjaer. 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (Oleaceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. Heredity 106, 788-797.

Raad voor plantenrassen. 2007: 8^e^ Rassenlijst Bomen. Eighth list of recommended species, varieties and provenances of trees for use in forest, landscape and urban areas. Ed. Buiteveld, J. CGN-WUR; 531 pp.

Queloz, V., C.R. Grünig, R. Berndt, T. Kowalski, T.N. Sieber & O. Holenrieder. 2011. Cryptic specification in *Hymenoscyphus albidus*. Forest Pathology 41, 133-142

Vereniging voor Natuurmonumenten. 2011: www.natuurmonumenten.nl/.../schimmel-tast-essen-in-kardinge-aan.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 249 – 261.

Ash DieBack (ADB) in amenity trees in the city of Amsterdam – development of a monitoring system and the first results of a large-scale survey in 2015

J. KOPINGA [1]*, S. M. G. DE VRIES [2]

[1] *Kopinga Boomadvies,* [2] *Alterra-Wageningen UR*
* *Corresponding author*: *j.kopinga1@chello.nl*

Abstract

During the month of June 2015 an extensive survey has been done regarding the state of the art of Ash Die Back (ADB) caused by *Hymenoscyphus fraxineus*, amongst the amenity trees in the municipality of Amsterdam, The Netherlands. The intention of the survey was to provide a basis for the future monitoring of the development of ADB and to test a monitoring system that was especially developed for this purpose.

One of the questions was whether the assessment of the general condition of the trees that was already used by the tree managers within the regular Visual Tree Assessment (VTA) would suffice or whether additional characteristics specifically focused on ADB also should be incorporated in order to increase the distinctiveness of the classification of the observations.

The observations are made on a sample of over 25 % (N = 4761) of the number of ash trees (N = 18477) that are registered in the urban tree management system of the city of Amsterdam.

Over 50% of the trees showed to be healthy and somewhat less than 50% showed a variable degree of twig dieback. For 37 % of the number of these trees (18% the total number of 4761 trees) ADB was designated as major cause of twig dieback and for 28% of the number of trees (14% of the total number) the primary cause of twig die back was ascribed to other factors. For 35% of the number of trees (17% of the total number) the main cause of twig die back remained uncertain.

The relatively high percentages respectively 28 and 35% can be explained by the influence of adverse growing site conditions that urban trees generally are exposed to and which negatively contribute to the health of many urban trees. This is also the reason why twig die back was a common phenomenon among amenity trees, especially trees standing in pavements, long before ADB was officially established in The Netherlands in 2010.

From the five tree species that were involved in the survey Fraxinus excelsior showed by far most of the twig die back caused by ADB. The ranking order of presence of ADB over the total number of sampled trees (N= 4761) was: *F. americana* (1,2%) < *F. ornus (4%)* < *F. angustifolia* (4,6%) < *F. pennsylvanica* (5,9%) < *F. excelsior* (21,2%).

Within the species *Fraxinus excelsior* (N=3998), the ranking order of the various cultivars, inclusive seedling trees, with twig die back caused by ADB (total number: 846) was: Hessei* (2,7%) < Doorenbos* (2,9%) < Diversifolia* (3,9%) < Atlas* (13,2%) < 'Unknown'* (17,9%) < Jaspidea (18,2%) < Westhof's Glorie (19,3%) < seedling trees (28,8%) < Allgold (32,0 %) < Eureka (33,3%). For cultivars marked with "*" the position in the ranking order is solely an indication for relative tolerance and may not be taken as an 'absolute' indication of susceptibility for ADB.

Trees standing in Forests as well as in plantings and sites with rough grass as ground vegetation are numerically more frequently infested by ADB than trees standing in paved or semi paved growing sites or frequently mowed lawns. This can possibly be explained by differences in infection pressure of the ADB fungus as a result of the influences of micro-climate or removal of leaf litter shortly after leaf fall in the autumn. Indications have been found that differences in infection pressure (c.q. infection biology) may influence the relative tolerance of ADB of the various species and cultivars c.q its position within the ranking of tolerance. This limits the possibilities to indicate an accurate 'absolute' tolerance on the basis of the results of this survey without data of subsequent surveys.

By including observation methods specifically aimed for the assessment of ABD such as determining the rate of crown die back, defoliation and formation of epicormic shoots, in the a more accurate distinction between classes of condition could be made than only by the assessment of condition as such. By incorporating these specific aspects in the monitoring system the development of ADB can be monitored with more accuracy in the future as well as it can be compared with the results of this large-scale survey. All of the additional ADB valuating aspects showed a very high and significant mutual correlation. Which combination of aspects will be the most distinctive in monitoring a trend of the development of ADB in the future will gradually become clear from the results of more monitorings during the next years.

With regard to the susceptibility of the various ash species and cultivars the findings of this survey are largely in agreement with the findings thus far elsewhere in the Netherlands and with the data in the international literature. There is no ground yet to change or adjust the practical guidelines for the management and control of ADB in practice.

Key words: Ash dieback, ADB, *Fraxinus*, *Hymenoscyphus fraxineus*, *Chalara fraxinea,* amenity trees, urban forest, monitoring.

1. Introduction

Infectious diseases on trees that attract the attention of tree managers over time are the Dutch Elm Disease (DED) and the Horse chestnut bleeding disease. The development of these are frequently monitored (usually each year) which provides insight in the progress or decline of the disease and the effect of control measures. Since a couple of years the development of the *Massaria* diseases of plane trees is monitored and from 2012 onwards also the ADB has drawn the attention of the tree managers because of its impact and rapid spread all over The Netherlands since 2010 (Bosschap, 2011, Lageschaar, 2012; VBNE, 2012; Kopinga & de Vries, 2012 a, b, 2015; Siebel & Reichgelt, 2013).

In order to monitor the development, and if possible to control, the disease at individual trees in the public urban environment during the coming years, the tree managers needed a system that was more or less comparable to the system used with the monitoring of the Horse chestnut bleeding disease. Also in the case of the ADB a so called ‘point-zero measurement’ was necessary.

One of the questions was whether the usual valuation of the tree's condition in the framework of the frequent VTA inspections (Visual Tree Assessment) would suffice for this purpose. And if not, which aspects were desirable to add to the observations according to some of the assessment systems that already were reported in the literature (Bakys, et al. 2013; Bakys, 2013; Enderle et al. 2014; Kirisits and Freinschlag, 2012; McKinney et al, 2011; Metzler et al. 2012; Pliura, et al. 2011, Skovsgaard et al. 2009; Stener, 2012). Within the framework of the point-zero measurement a number of valuation methods, or modifications to these methods have been incorporated into the set of observations of the assessment scheme for monitoring the development of ADB.

This article reports on the method that has been developed for this point-zero measurement and on a number of interesting results of the field observations in 2015.

2. Material and methods

2.1. The population of ash trees in Amsterdam

The number of amenity trees within the municipality of Amsterdam is not exactly known. Estimates vary from over half a million to one million. Many of the trees are standing in the 50 parks, park forests and recreational areas such as the Amsterdamse Bos (Amsterdam forest) with an estimated number of trees of about 105.000. At the moment the data of about 251.000 individual trees or groups of trees have been recorded in the tree management system of the city of Amsterdam. From this total 18447 (over 7% of the number of registered trees) are of the genus *Fraxinus*.

For the preparation of the monitoring the municipality has drawn up a particular kind of geographical information system (GIS) with the topographical position of all the ash trees together, including all different kinds of ash species and ash cultivars (see: http://maps.amsterdam.nl/essen/). Examples of this system are illustrated by the images 1 and 2. Comparable to the monitoring of the DED and the Horse chestnut bleeding disease data on the progression of ADB are also intended to be presented in this overview. An overview of the number of trees for each ash species is presented in table 1.

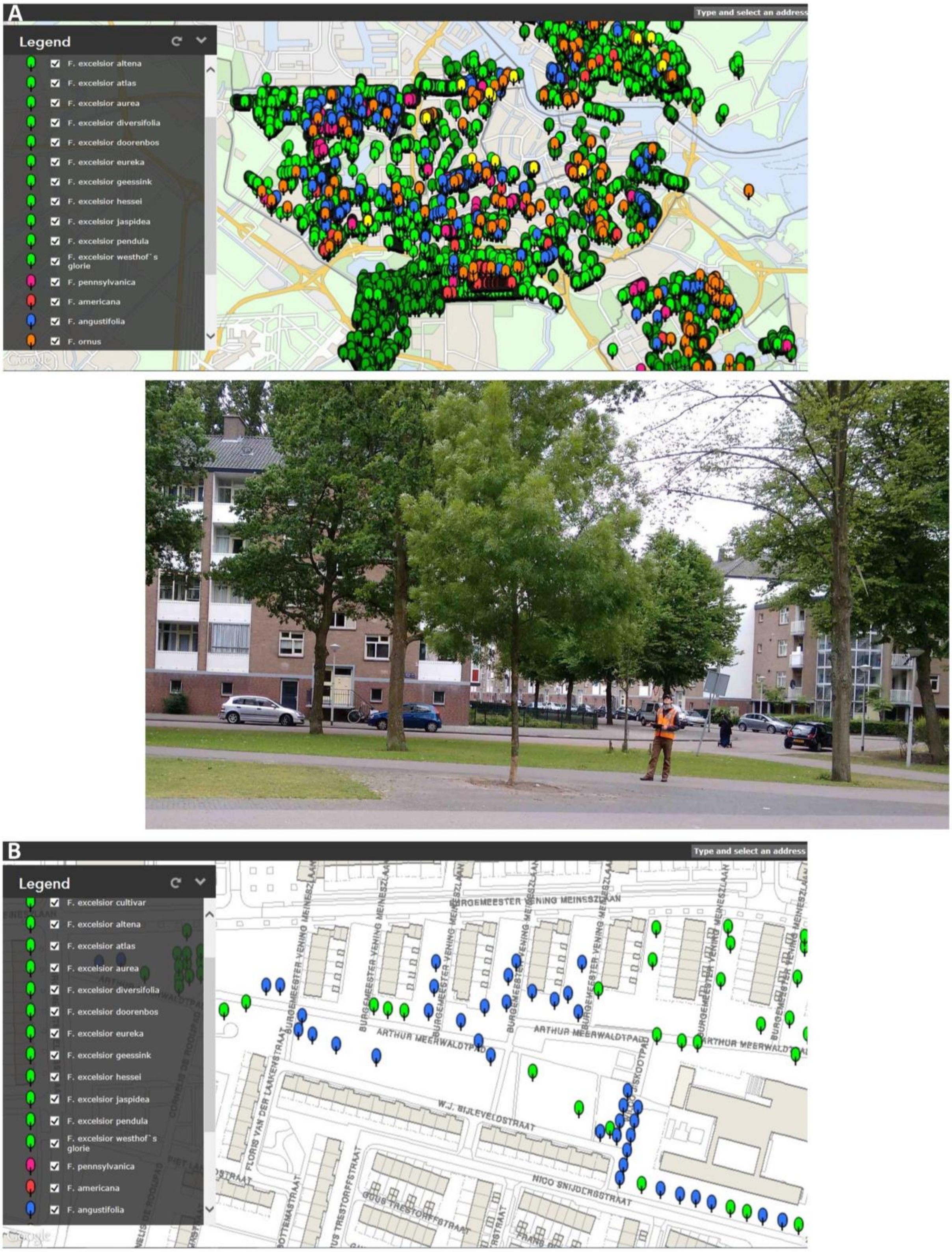


Image 1 A) Overview of a part of the number of ashes in Amsterdam that are registered as street-, avenue- or park trees. *Fraxinus excelsior*, both seedling trees and cultivars are indicated with a green colour, *F. pennsylvanica* with a purple colour, *F. americana* with a red colour, *F. angustifolia* with a blue colour, *F. ornus* with an orange colour and remainder species that are not common (such as *F. biltmoreana* and *F. xanthoxyloides*) with a yellow colour. (source: *http://maps.amsterdam.nl/essen/*). **B)** Detail of image 1.

Table 1 Overview per species of the number of registered ash trees.

Ash species	N-trees	% (of total N)
americana	321	1,7
angustifolia	829	4,5
biltmoreana	29	0,2
excelsior	16558	89,8
ornus	386	2,1
pennsylvanica	309	1,7
xanthoxyloides	1	0
Not indicated	14	0,1
Total	18447	100

2.2. Assessment method

As an approach the method for ADB assessment is comparable to the method of monitoring the Horse chestnut bleeding disease. This method is based on a visual assessment of various parameters of which the results can be entered in a spreadsheet (columns and rows) of a field computer. The recorded parameters are entered as nominal number or category code. Options such as category code, management data and other (binary) selections are already programmed in the spreadsheet and can easily be checked off during the observations. In extra columns remarks can be made, additional data can be entered or corrections can be put through if necessary.

The monitoring system (observation or assessment scheme) was developed in 2014 and previously tested in 2014 and 2015. A number of existing assessment schemes reported in the literature were selected and incorporated in the final set up. Before the survey the inspectors already were instructed and trained how to use the assessment scheme during a series of pilot studies in which individual trees were separately examined by each of the inspectors individually. Afterwards the mutual results were statistically analysed and the 'weak' points, showing significant differences were adjusted which significantly improved the uniformity and objectivity of the assessment.

The main data that are already present or can be entered during the observations are:

1. The unique ID number of the tree in the management system and topographical data such as city district, location (street) and GPS coordinates.

2. Tree species and cultivar. Missing data that could not be ascertained or corrected sufficiently during the inspections are marked as “unknown”. For the species *Fraxinus excelsior* is also indicated whether it's a seedling tree or a cultivar. This is not the case for the other *Fraxinus* species because from these species predominantly, if not exclusively only vegetative propagated commercially clones are planted. Cultivars that are not registered by a true name are marked as “cultivar”.

3. The growing stage of the trees expressed as:
Stem diameter category, incurring from 1-10 cm (= class 1) to > 80 cm (= class 9);
Tree height category, incurring from 0-6 meter (= class 1) tot > 24 m (= class 7);
Crown diameter category, incurring from 0-5 meter (= class 1) to > 20 m (= class 5).

4. Growing site, divided in six categories: 1 = Forest, 2 = Small forest like plantings (hedgerows etc.), 3 = Rough grass vegetation (not mowed), 4 = lawn (frequently mowed), 5 = semi paved area and 6 = paved area (sidewalks)

5. The all-over condition of the tree, distinguished into seven classes, incurring from excellent (class 1) to good (class 2), fair (class 3), moderate (class 4), poor (class 5), very poor (class 6) and dead (class 7).

6. Presence of ADB, noted as 0 = No or 1 = Yes.

7. The accuracy of the assessment of infestation by ADB as primary factor of decline of the tree, noted as 0 = “No ADB”, 1 = “Predominantly ADB” and 2 = “Uncertain”

8. Date of inspection, noted as number (Julian day).

9. Name of the observer.

10. Remarks on specific phenomenons such as other diseases caused by biotic and abiotic factors (as text in for this purpose designated columns).

11. An estimate of the year of first infestation by ADB.

12. An indication of the phase of infestation: 1 = petiole, 2 = rachises, 3 = leaf withering, 4 = Twig die back.

13. A 'global' score ("P-score") indicating the degree of die back of the tree, based on the scheme and characteristics reported by Pliura et al (2011). In the monitoring system the score is used in reverse order: 0 = No twig die back and 6 = Death of the whole tree.

14. A score ("K-score") indicating the degree of defoliation of the tree, based on the system reported by Kirisits & Freinschlag (2012). The score is expressed as average percentage defoliation of the whole tree crown.

15. A score for the presence of epicormic shoots in the tree crown, based on the system reported by Enderle et al. (2014) with some modifications. The score is expressed as percentage of the whole tree crown.

16. An estimate of the largest diameter of dead wood (in cm) present in the tree crown.

Additional digital photographs are made of each inspected tree and numbered in for this purpose designated columns.

2.3 The survey

A large-scale monitoring with the use of this particular scheme was performed in June 2015 by the Bomendienst van het Bureau voor Tuin- en Landschapsverzorging (BTL) in cooperation with Alterra Wageningen UR and the Centre for Genetic Resources (CGN Wageningen UR). The survey was carried out in the period between June 3d and July 1st 2015 by three inspectors (examiners A, B and C) operating individually. All three inspectors are skilled arborists (level European Tree Technician).

The assessments were made on a selected sample of 4783 ash trees (= over 25% of the total population of registered ashes in Amsterdam) in which the trees were examined on all of the aspects described above. The data of 4761 ashes were qualified for further statistical processing and analysis.

The choice for a selected (and not a random) sampling was based on logistical reasons and limitations where aspects such as reachability and accessibility of the objects within an acceptable period of time were leading. Especially objects in which relatively many trees, preferably various ash species with various age, and standing on various growing sites were qualified in first instance for further inspection.

The data were analysed using various statistical software (JASP, PAST, SPSS). Because most of the data and recorded variables of the observed aspects were not normally distributed, the statistical tests for differences between the averages of categories and classes were predominantly made using non parametric methods such as the Kruskal-Wallis test with Mann-Whitney, Bonferroni posthoc. Unless otherwise indicated, a p-value of 0.05 is taken as level for statistical significance.

3. Results

3.1. General

The total number of ash species on each growing site is presented in table 2. Because of the small number in which *F. biltmoreana* is present in the survey, this species has been omitted for further statistical processing and analysis. The average stem diameter (DBH) class for each species is presented in table 3.

Table 2 Overview of the number and numeric percentage of ash species (with in the first column the percentage of the number of the species on the total number of ashes) on each of the growing sites.

	Growing site						
Ash species	Hedgerow	Lawn	Gravel	Pavement	Gras	Forest	Total
americana	209	9	0	30	1	0	249
5,2%	83,9%	3,6%	0,0%	12,0%	0,4%	0,0%	100%
angustifolia	30	71	21	122	9	8	261
5,5%	11,5%	27,2%	8,0%	46,7%	3,4%	3,1%	100%
biltmoreana	1	2	0	0	1	0	4
0,1%	25,0%	50,0%	0,0%	0,0%	25,0%	0,0%	100%
excelsior	523	1302	67	355	1115	636	3998
84,0%	13,1%	32,6%	1,7%	8,9%	27,9%	15,9%	100%
ornus	47	91	1	51	5	3	198
4,2%	23,7%	46,0%	0,5%	25,8%	2,5%	1,5%	100%
pennsylvanica	1	11	6	14	19	0	51
1,1%	2,0%	21,6%	11,8%	27,5%	37,3%	0,0%	100%
Total	811	1486	95	572	1150	647	4761
100%	17,0%	31,2%	2,0%	12,0%	24,2%	13,6%	100%

Table 3 Average DBH class per ash species. The column "Sig. 0.05" indicates significant differences between the average DBH class. Figures with the same letter are not significantly different.

Ash species	N	Average DBH-class	Sig. 0.05
americana	249	1,22	a
angustifolia	261	2,51	bc
excelsior	3998	3,88	d
ornus	198	2,08	b
pennsylvanica	51	2,78	c
Total	4757	3,58	n.a.

3.2. Twig die back and infestation by ADB.

Over 50% of the number of trees (all species) looked healthy and a little less than 50% showed some degree of twig die back. For 37 % of the number of trees with twig die back (18% of the total number) ADB was assigned as being the predominant cause. For 28% of the number of trees with twig die back (14% of the total number) the cause was ascribed to other factors. For 35% (17% of the total number) it remained uncertain if ADB was present and therefore if, and to what extent, twig die back was caused by ADB. An overview of the degree of twig die back per species and the degree in which the cause could be ascribed to ADB is presented in figure 1.

When only the presence of twig die back is regarded, despite of its cause, the results can be ranked by the following incurring order: *F. americana* (a) < *F. pennsylvanica* (ab) < *F. angustifolia* (b) < *F. ornus* (b) < *F. excelsior* (c).

The ranking order of the numeric percentage in the category "predominantly ADB" with regard to "No ADB" (the difference between the left and right bar) is: *F. americana* (a) < *F. pennsylvanica* (ab) < *F. ornus* (ab) < *F. angustifolia* (b) < *F. excelsior* (c). The letter behind the species indicates the significance of differences between the species. Species with the same letter do not differ significantly.

3.3. General condition, P-score, K-score, Diameter of dead branches and presence of epicormic shoots.

In order to determine whether apart from the valuation of the general condition of the tree, the valuation of additional aspects may be indicative for the degree of twig die back (which then would give more distinctive differences between the various categories of either the general condition or specifically twig die back), calculations are made on the total number of trees (all species together) and separately for only the species *F. excelsior*. An overview of the results of all trees and all species (N = 4757) is presented in table 4.

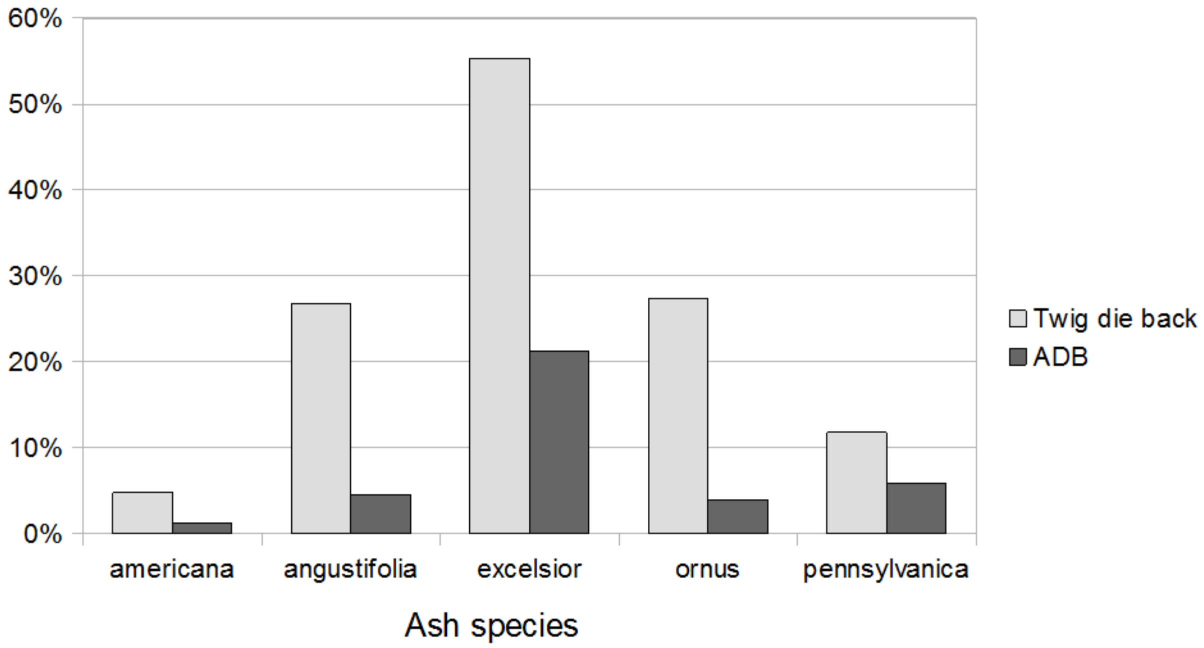


Figure 1 Overview per species of the number of trees (as percentage of the total number of observed trees for the particular species) showing twig die back and the number of trees (also as percentage of the total number of observed trees) of which the cause of twig die back was ascribed to ADB.

Table 4 Overview of the average twig die back (as percentage of the number of trees presented in column "N") and the P-score, K-score, Deadwood diameter (DWD, in cm) and the presence of epicormic shoots in the whole tree crown (as percentage) for each of the condition classes. The column "Sig." indicates significance of differences of the average values in each column. Values with the same letter are not significantly different. "KW test stat." indicates the statistics of an Kruskal-Wallis test, "F-anova" is the F-value of an ANOVA test (where in this case the values of the categories are regarded as continuous numbers).

Condition	N	Twig die back	Sig.	P-score	Sig.	K-score	Sig.	DDW	Sig.	Epic. Shoot	Sig.
Excellent	313	12%	a	0,11	a	0,3	a	0,1	a	0,9	a
Good	3098	38%	ab	0,26	a	1,1	a	0,4	a	4,4	a
Fair	957	78%	bc	0,59	a	5,7	a	1,0	ab	14,7	a
Moderate	272	96%	c	1,37	b	27,0	b	1,9	b	38,9	b
Poor	88	100%	c	2,68	c	64,8	c	3,1	c	69,2	c
Very poor	30	93%	c	3,37	d	82,3	d	4,2	c	84,3	c
Dead	3	100%	c	3,33	cd	66,7	c	8,0	d	0,0	a
Total	4761	49%		0,44		5,2		0,6		9,9	
KW test stat.		981		1028		1133		1100		979	
F Anova		206		596		1311		355		507	

It appears that only the presence of twig die back does not give a clear distinction between the condition classes 3 to 7 (respectively "fair", "moderate", "poor", "very poor" and "dead" while both the P-score and K-score do give a significant distinction between those classes (apart from the class "dead", because of the small number of trees present in this class). This also accounts to a lesser extent for the parameters: Dead wood diameter and epicormic shoots. The K-score is the most determining for the distinction between condition classes when analysed by a discrimination analysis (on a pooled correlation of 0,994 of all variables together). But using the K-score results in a substantial number of outliers within the "better" condition classes (see figure 2b). However it cannot be excluded that some of the outliers might be the result of an abusive import of the data in the spreadsheet of the field computer during the observations. The backgrounds of this deviation may become more clear by analysing the results of subsequent monitoring.

Otherwise the mutual correlation of the added parameters and also the correlation of these parameters with twig die back are all very significant at p = 0,000 (Spearman's test) with the highest correlation (Rho = 0,955) between the P-score and K-score (see table 5).

Table 5 Correlations (Spearman's Rho rank correlation) between the parameters: Condition, Twig die back, P-score, K-score, Diameter dead wood (DDW) and percentage epicormic shoots in the tree crown. The correlations are all calculated from the total number of trees (N = 4761).

	Twig die back	P-score	K-score	DDW	Epic. Shoot (%)
Conditie	,452**	,412**	,445**	,444**	,408**
% Twig die back(Yes/No)	1	,715**	,732**	,770**	,479**
P-score		1	,955**	,895**	,666**
K-score			1	,917**	,729**
DDW				1	,670**
Epicormic shoots (%)					1

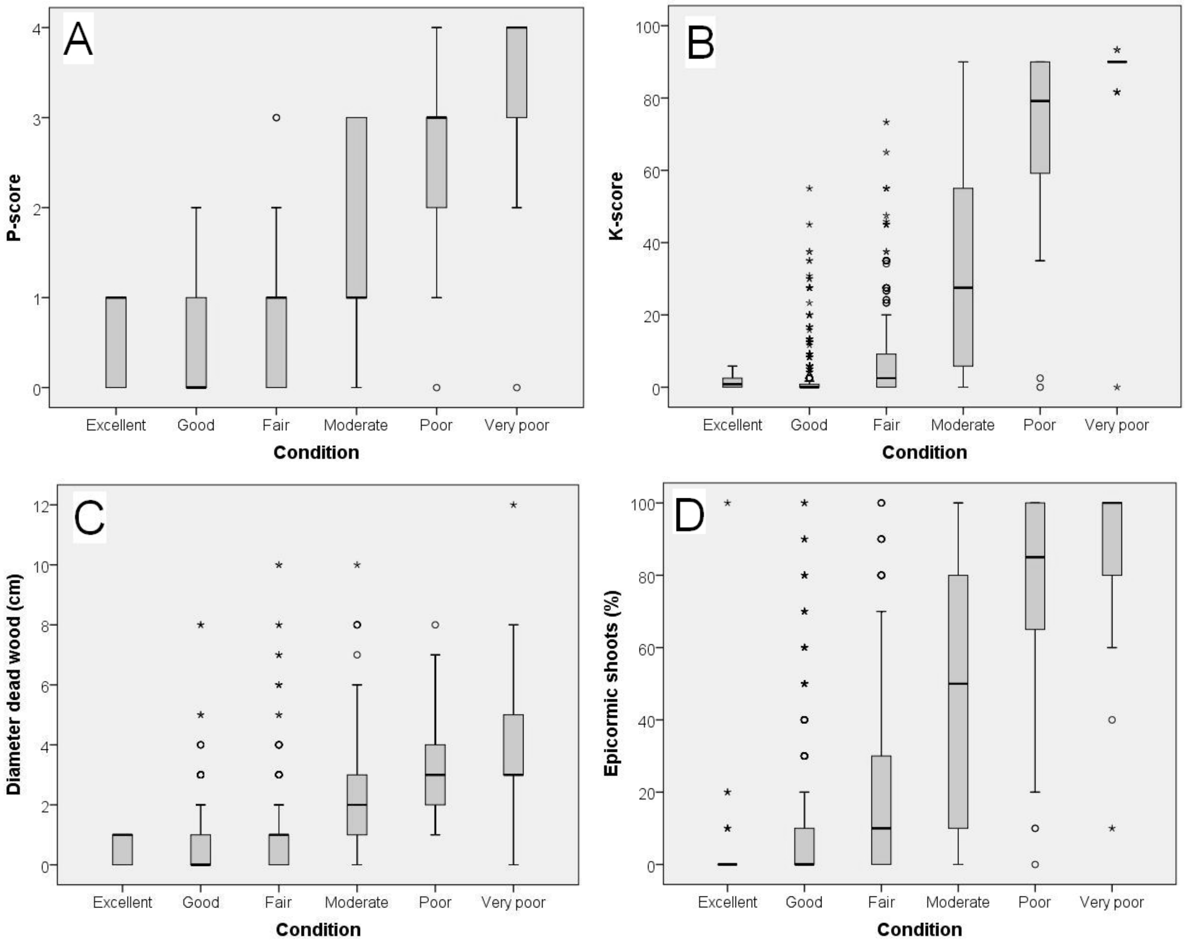


Figure 2 Boxplots of the relation of the general condition of the trees with **A)** the P-score, **B)** the K-score, **C)** the maximal diameter of dead branches and **D)** the percentage of epicormic shoots in the tree crown.

3.4. Calculations on *Fraxinus excelsior*

Not every ash species is more or less evenly present on the various growing sites (see table 2). Therefore the environmental and biological conditions for the infestation of ADB may be variable, which influences the possibilities for mutual comparison of the ash species. But also the degree of twig die back caused by other agents such as suboptimal growing site factors may vary amongst the various growing sites. Also there are substantial differences between average stem diameter (DBH) between the species (see table 3). And also not all species are evenly examined by all three of the inspectors. This limits the possibilities of a “fair” statistical comparison. Therefore the following calculations and statistical analyses have been restricted to the species

Fraxinus excelsior which species by number is broadly present on all growing sites (see table 2) and is also more or less evenly examined by all of the inspectors. Besides, as species it outnumbers by far the presence of other species: almost 90% of all the ashes in Amsterdam is common ash (see table 1). An overview of the number of cultivars of *F. excelsior* on each of the distinguished growing sites is presented in table 6.

Table 6 Overview of the number and numeric percentage of cultivars of *F. excelsior* on each of the distinguished growing sites.

	Growing site						
Cultivar	Hedgerow	Lawn	Gravel	Pavement	Grass	Forest	Total
Unknown	36	166	3	11	25	88	329
8,2%	10,9%	50,5%	0,9%	3,3%	7,6%	26,7%	100,0%
Seedling	178	148	21	79	477	366	1269
31,7%	14,0%	11,7%	1,7%	6,2%	37,6%	28,8%	100,0%
Skyline	1	2	0	15	3	3	24
0,6%	4,2%	8,3%	0,0%	62,5%	12,5%	12,5%	100,0%
Allgold	12	1	0	1	8	3	25
0,6%	48,0%	4,0%	0,0%	4,0%	32,0%	12,0%	100,0%
Altena	0	6	0	2	0	0	8
0,2%	0,0%	75,0%	0,0%	25,0%	0,0%	0,0%	100,0%
Atlas	4	73	0	25	7	5	114
2,9%	3,5%	64,0%	0,0%	21,9%	6,1%	4,4%	100,0%
Aurea	0	3	0	0	0	0	3
0,1%	0,0%	100,0%	0,0%	0,0%	0,0%	0,0%	100,0%
Diversifolia	20	51	0	26	1	4	102
2,6%	19,6%	50,0%	0,0%	25,5%	1,0%	3,9%	100,0%
Doorenbos	13	29	12	5	11	0	70
1,8%	18,6%	41,4%	17,1%	7,1%	15,7%	0,0%	100,0%
Erosa	2	2	0	0	1	0	5
0,1%	40,0%	40,0%	0,0%	0,0%	20,0%	0,0%	100,0%
Eureka	2	12	0	14	11	0	39
1,0%	5,1%	30,8%	0,0%	35,9%	28,2%	0,0%	100,0%
Geessink	0	9	0	0	9	0	18
0,5%	0,0%	50,0%	0,0%	0,0%	50,0%	0,0%	100,0%
Hessei	19	1	0	10	7	0	37
0,9%	51,4%	2,7%	0,0%	27,0%	18,9%	0,0%	100,0%
Jaspidea	17	8	0	46	5	1	77
1,9%	22,1%	10,4%	0,0%	59,7%	6,5%	1,3%	100,0%
Veltheimii	0	0	0	0	5	0	5
0,1%	0,0%	0,0%	0,0%	0,0%	100,0%	0,0%	100,0%
Westhof's Glorie	219	791	31	121	545	166	1873
46,8%	11,7%	42,2%	1,7%	6,5%	29,1%	8,9%	100,0%
Total	523	1302	67	355	1115	636	3998
100,0%	13,1%	32,6%	1,7%	8,9%	27,9%	15,9%	100,0%

3.5. Twig die back and ADB on *Fraxinus excelsior*

Approximately 45 % of the number of trees (all cultivars) was healthy and over 55 % showed some degree of twig die back. For 38% (= 21% of the total number of trees) the cause of twig die back was ascribed to ADB. For 26 % (14,5 % of the total number of trees) the main cause was ascribed to other factors and for 35% (19,6% of the total number) it remained uncertain if ADB was the main cause of the twig die back. An overview of the numeric percentage of trees showing twig die back and the percentage for each cultivar is presented in figure 3 where cultivars that were present in a number less than 15 have been omitted.

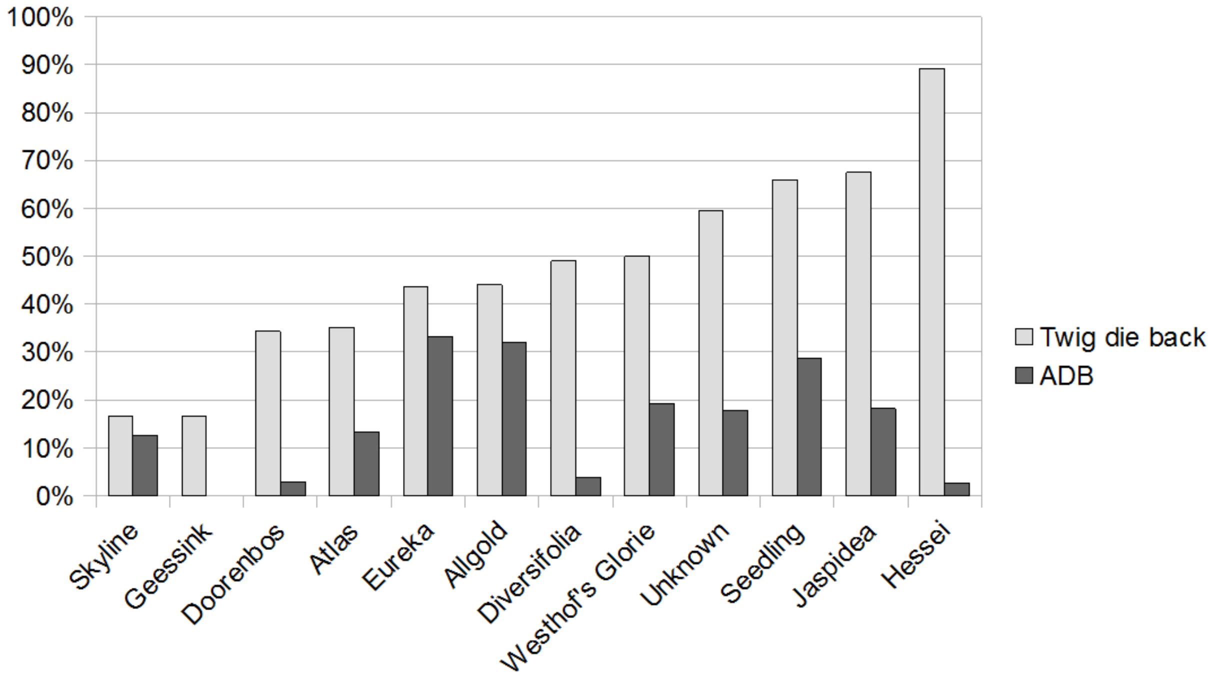


Figure 3 Overview per cultivar of *F. excelsior* of the numeric percentage (of the total number of each cultivar) showing symptoms of twig die back (left bar) and the numeric percentage of trees were the main cause of twig die back was ascribed to ADB (right bar).

On the basis of the percentages within the category “predominantly ADB” relative to the category “other factors” the following ranking order can be drafted: Geessink < Hessei < Doorenbos < Diversifolia < Skyline < Atlas < 'Unknown' < Jaspidea < Westhof's Glorie < Seedling trees < Eureka. The small number of trees of a particular cultivar in the category “predominantly ADB”, together with the number of these cultivars on the various growing sites limits the possibilities for an accurate statistical analysis and therefore the results are not presented here.

3.6. Calculations on seedling trees and the cultivar Westhof's Glorie of *F. excelsior*

Within the population of trees of the species *F. excelsior* the seedling trees (“S”, N = 1269) and the cultivar Westhof's Glorie (“WG”, N = 1873) are by far the most represented (respectively 32 en 47 % of the total number of trees of *F. excelsior*). Because both groups or 'cultivars' are present on all growing sites and have been examined more or less evenly by all three of the inspectors the calculations presented below have been restricted to these two groups of trees. Calculations have been made on the degree of presence of twig die back and ADB on the various growing sites and the intensity of infestation by ADB and the DBH class of trees that were most infested (all expressed as numeric percentage).

3.7. Twig die back on each of the growing sites

The numeric percentage of trees showing symptoms of twig die back and the percentage of trees where ADB was ascribed as main cause for both of the groups together (S + WG) is presented in figure 4.

The difference in percentages of twig die back between S and WG over all growing sites together are significant (Mann-Whitney p = 0,000). On the growing sites “Lawn”, “Gravel”, “Grass” and “Hedgerow” the percentages of twig die back differ significantly between S and WG, (at p = 0.05). On the other growing sites (respectively “Forest” and “Pavement” there are no significant differences between S and WG. By comparing the relation between the percentages of “predominantly ADB” and “No ADB”, The following ranking order can be drawn based on the number of trees per type of growing site where ADB was ascribed as the main cause of twig die back: Grass (not mowed) (a) > Forest (ab) > Hedgerows (ab) > Lawn (abc) > Gravel (bc) > Pavement (c). The letter behind the species indicates the significance of differences between the species. Species with the same letter do not differ significantly.

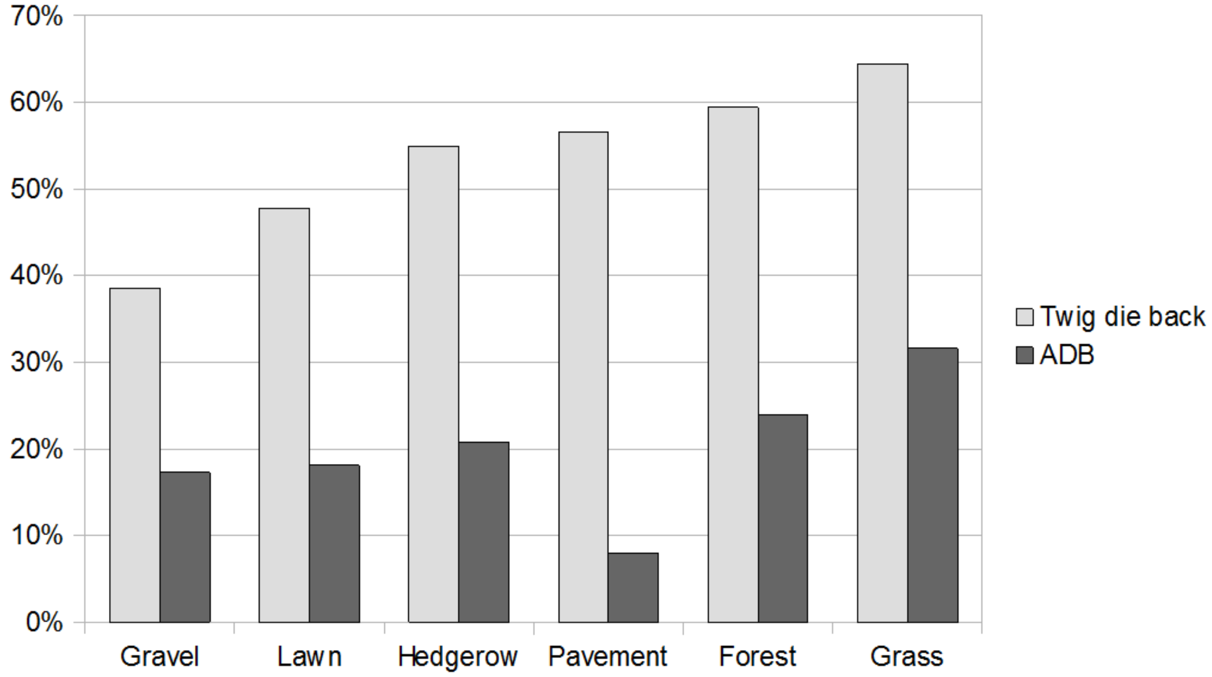


Figure 4 Percentage (of the total number of S and WG together) of the number of trees with symptoms of twig die back and the percentage of trees where the main cause of twig die back was ascribed to ADB on each of the growing sites.

4. Discussion and conclusions

4.1. Assessment of ADB on the basis of the general condition of the trees

The result of the survey show clearly that urban trees are prone to multiple factors that apart from infestation of ADB negatively influence the general condition of the trees and may result in twig die back. This impedes that the assessment requires a high level of expertise of the inspectors. During the previous pilot studies for testing the monitoring system mutual 'tuning' about the way how inspectors must observe and examine trees for the presence of ADB appeared to be highly desirable if not a compelling necessity.

Some comments can be put to the question whether the monitoring of ADB could be based solely on the assessment of the general condition of the trees:

Although the correlation (Spearman's Rho = 0.425) between condition and twig die back is highly significant (see table 5), it is despite this significance still lower than it could be expected or desired. It indicates a substantial amount of overlap between the categories as well as a substantial number of outliers. Which on its turn decreases the distinctiveness. Also the correlation with inspection methods that are more specifically aimed at the assessment of ADB (P-score, K-score, diameter of dead wood and percentage of epicormic shoots in the tree crown) is remarkably lower than the mutual correlation between the specific methods. This is partly explainable for obvious reasons: For the valuation of the tree's condition not only the presence of twig die back is regarded, but also aspects such as annual shoot length, leaf size, leaf colour, transparency of the crown, et cetera. Not all of these aspects are directly related to the effects of infestation of ADB. And because of all this it is obvious that trees with only a minor degree of twig die back (and receive the rating “Yes” for presence of ADB) get a score of “good” or even ”excellent” when evaluated for their general condition. As an example for this, it appears that the numeric percentage of trees that are infested by ADB within the category “excellent” is higher than the percentage in the category “good”.

Although the infestations of ADB in these categories predominantly are of a low level without any significant effect on the tree's condition, it implies that the presence of ADB cannot be established sufficiently only on basis of the assessment and monitoring of the condition of the trees. In this respect it is preferable to combine the valuation of the condition with one or more of the other methods for assessing the effect of ADB.

On the basis of a discriminant analysis the K-score appears to be the most suitable, followed by the P-score as a 'good' second choice (also because of the strong correlation of R = 0,955) between the results of both methods. However the tree manager will also be interested in the future formation of dead branches in the tree crown (because for reasons of traffic safety), so this aspect cannot be omitted either. What will be the 'best' combination of methods cannot be exactly pointed out on the basis of the first results of the reported survey reported in this paper. For this purpose at least one, or preferably more, repetitions of this survey during the next few years are necessary.

4.2. Susceptibility and tolerance of species and cultivars.

The problem that it is often not exactly clear to indicate which factors are the main cause of twig die back (ADB or not) makes it difficult to give more than just general information about the susceptibility for ADB of the various species and cultivars.

Among others this is illustrated by the relatively high degree of twig die back of cultivars such as Hessei (of *F. excelsior*) from which only one tree out of 37 was actually indicated to be infested by ADB as the cause of twig die back. However, this cultivar is known already for a long time to perform poor in an urban environment, especially when planted in paved areas such as sidewalks. Knowing this, it is not so much the susceptibility for ADB to discourage its use, but more the general usability under 'harsh' city environments.

Another remarkable result is the frequency of which twig die back of the species *F. ornus* is noted as “predominantly ADB” (8 times out of 198 trees, of which 27 are noted as “uncertain”). This is remarkable, because most of the literature thus far reports the species as being not susceptible for ADB, or anyway to be very tolerant (Lösing, 2013). In order to give more information about arising questions on the susceptibility of *F. ornus* for ADB, further diagnostic research is required. However, this lies outside the scope of the future monitoring.

Because of the relative small number where twig die back of trees from the species *F. americana, angustifolia* and *pennsylvanica* is noted as “predominantly ADB” or “uncertain”, it is not possible to give sufficient reliable information on the 'absolute' degree of susceptibility of these species and neither in relation to the susceptibility of *F. excelsior*.

In spite of the mentioned limitations for mutual comparison of susceptibility, and although a couple of cultivars show some deviation from the general pattern, the findings of this survey on the susceptibility of cultivars of *F. excelsior* are generally in agreement with the reported data thus far from both Dutch as well as from foreign studies (Lösing, 2013, De Vries & Kopinga, in this issue).

4.3. Impact of growing site factors

Another limiting factor to underpin the distinction in apparently differences in susceptibility is the fact that some species are mostly, if not exclusively present as street tree or in parks with mowed lawns. This may imply that besides genetically determined susceptibility also differences in infection pressure are influencing the results. On the one hand because of the shed leaves, which includes the fruiting bodies of the fungus that develop on these leaves, are not removed, while this usually is the case where trees are standing in pavement or semi-pavement such as gravel, and on the other hand, there also may be differences in the infection biology of the fungus because of differences in micro-climate on the particular growing sites. As an average, street trees are standing in a warmer and dryer environment. All these aspects, together with any other site factor that affect the health of the trees distorts or limits statistical comparison.

4.4. Consequences for the management of ash trees

The survey reported in this paper concerns a point-zero measurement of which at this moment no trends in future development can be indicated. Therefore no evidence could be derived that could serve as a basis for adjusting the guidelines for tree management published thus far in The Netherlands (Kopinga & de Vries, 2015). Anyway, the results appear to confirm the findings reported in the literature and also the findings of observations in practice. This accounts for both the susceptibility of the various species and cultivars as well as the impact of growing site (forest versus urban green) on the development of ADB (R. Kehr, pers comm.). For the urban forest it is strongly advised to refrain from planting species and/or cultivars of ash trees that are highly susceptible for

ADB. There's no convincing evidence yet to change the conventional management such as pruning of street trees with regard to control ADB. The extent of formation of dead branches in the tree crowns caused by ADB (these branches have to be pruned because of reasons for traffic safety) have to become apparent from future observations.

For large areas of forest plantings it should still be considered to what extent transformation to other forest types or guiding artificial regeneration with more ADB tolerant seedlings could be a practicable option.

5. References

Bakys, R. Vasaitis, P. Barklund, K. Ihrmark & J. Stenlid. 2009. Investigations concerning the role of *Chalara fraxinea* in declining *Fraxinus excelsior*. Plant Pathology 58, 284-292.

Bakys. R. 2013. die back of *Fraxinus excelsior* in the Baltic Sea Region – Associated Fungi, Their Pathogenecity and Implications for Silviculture. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala – Sweden.

Bosschap. 2011: www.bosschap.nl/cms/.../8.../Praktijkadvies%20essentaksterfte.pdf.

Enderle, R., A. Nakou, K. Thomas & B. Metzler. 2014. Susceptibility of autochthonous German *Fraxinus excelsior* clones to *Hymenoscyphus pseudoalbidus* is genetically determined. Annals of Forest Science. Published on line (DOI 10.1007/s13595-014-0413-1). 11p.

Kirisits, T. & C. Freinschlag. 2012. Ash die back caused by *Hymenoscyphus pseudoalbidus* in a seed plantation of *Fraxinus excelsior* in Austria. Journal of Agricultural Extension and Rural Development Vol. 4(9), pp. 184-191.

Kopinga, J. & S.M.G. de Vries. 2015. Essentaksterfte, Bosbouwkundig beheersbaar? Bosberichten 2015#1. Stichting Probos, Wageningen. 4p.

Kopinga, J. & S.M.G. de Vries. 2013a. Onderzoek naar de essentaksterfte – De internationale ontwikkelingen en stand van zaken. Boom-In-Business 9: 72-75.

Kopinga, J. & S.M.G. de Vries. 2013b. Differences in susceptibility to Chalara fraxinea (twig die back of ash) of selections of Common ash (Fraxinus excelsior) in The Netherlands – Report of the observations and results of 2012. Report nr. 26. Centrum voor Genetische Bronnen Nederland (CGN), Wageningen, Wageningen UR (University & Research centre). 31 p.

Lageschaar, M. 2012. Verspreiding van essentaksterfte: wat staat ons te wachten? Vakblad Natuur Bos en Landschap mei 2012: 21-23.

Lösing, H. 2013. Susceptibility of *Fraxinus* species to ash die back caused by *Chalara fraxinea*. Acta Horticulturae 1014: 201-203.

Mc Kinney, L.V., L.R. Nielsen, J.K. Hansen & E.D. Kjaer. 2011. Presence of natural genetic resistance in *Fraxinus excelsior* (Oleaceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. Heredity 106, 788-797.

Metzler, B., R. Enderle, M. Karopka, K. Töpfner & E. Aldinger. 2012. Entwicklung des Eschentriebsterbens in einem Herkunftsversuch an verschiedenen Standorten in Süddeutschland. Allgemeneine Forst- und Jagdzeitung 183 (7/8): 168-180.

Pliura, A., V. Lygis, V. Suchockas & E Bartkevicius. 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 Vol. 17, no. 1 (32): 17-34.

Siebel, H. & A. Reichgelt. 2013. Praktijkadvies essentaksterfte. Bosschap, bedrijfsschap voor bos en natuur, Driebergen. 5p.

Skovsgaard, J.P., I.M. Thomsen, I.M. Skovgaard & T. Martinussen. 2009. Associations among symptoms of die back in even-aged stands of ash (*Fraxinus excelsior* L.). Forest Pathology, 40: 7-18.

Stener, L-G. 2012. Clonal differences in susceptibility to the die back of *Fraxinus excelsior* in southern Sweden. Scandinavian Journal of Forest Research 2012: 1-12.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 262 – 272.

Ash Dieback – a continuing threat to veteran ash trees?

V. Bengtsson*[1], Anna Stenström[2]

[1] Pro Natura, [2] County Administrative Board of Västra Götaland
** Corresponding author: vikki.bengtsson@pro-natura.net*

Abstract
Ash dieback (*Hymenoscyphus fraxinus*) is a fungal disease which affects ash throughout Sweden. Monitoring to study of the impact of ash dieback on veteran trees were made in the west of Sweden in 2009, 2011, 2013 and 2015. The study found that 90% of the ash trees observed were affected by ash dieback disease in 2015 compared with 62% in 2009 and that 35 ash trees have died (11%) since the monitoring began. In 2009 there was no relationship between girth and ash dieback, but in 2015 the correlation between girth and the impact of ash dieback was statistically significant and no trees under 140cm in girth were free of ash dieback. The results in relation to the effect of ash dieback on pollarded trees varied between the years studied. Data from all four monitoring occasions show that maiden trees were significantly more affected than lapsed pollards.

Key words: Ash dieback, pollards, veteran trees, *Hymenoscyphus pseudoalbidus,* ash

Introduction

Ash dieback (*Hymenoscyphus fraxinus*) is a fungal disease which affects ash throughout Sweden and the disease was first recorded in 2001 (Barklund, 2009). According to a national study of random plots in the area of Götaland, which was carried out by SLU in 2009 and 2010, some 30% of all ash trees larger than 10cm in diameter were heavily affected or dead (www.skogskada.slu.se). In Sweden, a great deal of money is invested in pollarding ash trees (Rural Development Programme) and also for the restoration of old lapsed pollards. In addition, veteran ash trees are important cultural history elements in the Swedish landscape and bearers of a wide range of biodiversity. Ash was put on the Swedish Red List in 2010 (Gärdenfors, 2010; Artdatabanken, 2015). There is a long list of threatened species entirely dependent on ash in Sweden such as *Agrilus convexicollis*, *Pyrenula nitidella*, *Perenniporia fraxinea* and *Euphydryas maturna*. Many other species use ash as the main substrate and the Swedish Species Information Centre has identified 180 Red Listed species associated with ash. Prognoses from research from Roberge et al, 2011 has shown that the moss *Neckera pennata* is likely to reduce significantly as a result of ash dieback. There is also a risk that the tree species that will replace ash (and also elm) when they die will not have the same bark characteristics as ash. This will lead to a further reduction in the habitat for species associated with trees with a high pH (Jönsson & Thor, 2012). Even if some ash trees appear to have a greater level of resistance to ash dieback, the disease remains a great threat to the ash population in Sweden not only as it can kill trees itself, but also because landowners fell ash trees due to a lack of information or because they believe that they then reduce the risk of spread.

A fifth of the ash trees in Sweden are found in Västra Götaland (Skogsdata, 2012) and therefore it is a useful study area. There is relatively little scientific evidence relating to ash dieback and pollards, or indeed the impact of ash dieback on veteran ash trees in general. Due to the lack of data, relating to pollards and veteran ash trees, a monitoring programme was established in 2009 by the County Administrative Board of Västra Götaland (Bengtsson & Stenström, 2009). The aims of the monitoring were to obtain an overview of how ash dieback was affecting the county's veteran ash trees and to be able to follow the development of the disease over the coming years. The same trees were visited in 2009, 2011, 2013 and 2015 (Bengtsson & Stenström, 2009; Bengtsson et al, 2012; Bengtsson, 2014; Bengtsson & Stenström, 2014; Bengtsson, 2016). This paper describes the results from all of these survey occasions.

Method

In 2009 around half of the County of Västra Götaland had been surveyed to record veteran trees and the database contained information on more than 25,000 trees, of which 17% were ash. A random sample of 330 of these trees, both pollards and maidens, were examined in the summer of 2009 and re-visited in the summers of 2011, 2013 ad 2015 (Figure 1). Among the group of trees that were recorded as pollards, there was an even spread between those which had been recently pollarded (within the last ten years) and lapsed pollards (more than thirty years since they were last pollarded). The ash trees were located with the help of a GPS and map.

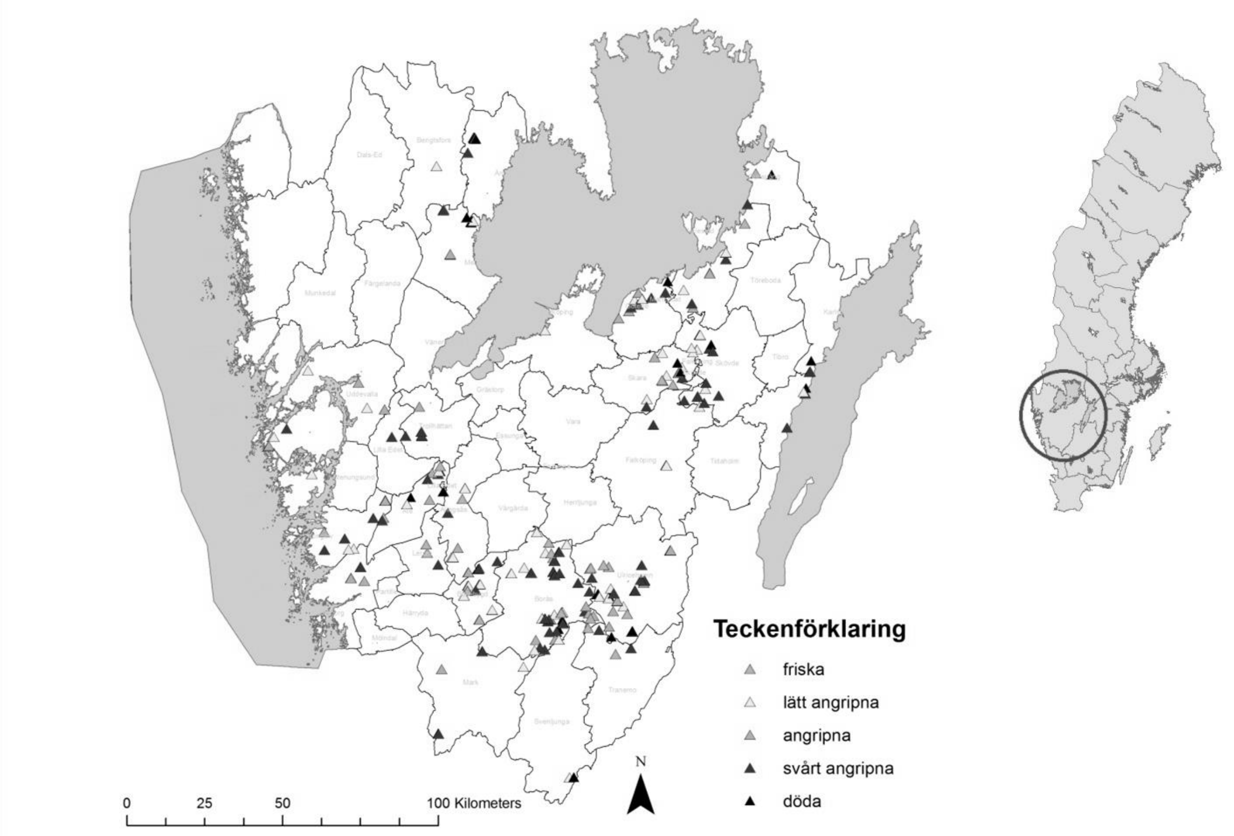


Figure 1 Map showing the location of the ash trees that have been monitored and the location of the County of Västra Götaland in Sweden (inset). Symbols from the top to bottom: “friska” – healthy; “lätt angripna” – slightly damaged;”angripna” – damaged; “svårt angripna” – severely damaged; “döda” – dead.

The trees were assessed from the ground with the help of binoculars, using field symptoms of ash dieback such as red/brown shoots and necroses as indicators. No laboratory analyses were carried out. Health assessed in the field (i.e. level of damage) has been shown by McKinney *et al*. (2011) to strongly correlate with the abundance of necroses and has thus been judged as a reliable method to assess the impact of *H. fraxineus* on ash trees. In this method the trees were scored according to a five-point scale:

- 0 – completely healthy.
- 1 – lightly affected (c.<10 % of the crown affected).
- 2 – affected (c.10-30 % of the crown affected).
- 3 – significantly affected (c.>30% of the crown affected).
- 4 – dead.

The differences between years and the effects of pollarding were analysed using repeated measures ANOVA, after checking for normal distributions and equal variances and correcting the p-values with Huynh-Feldt correction for “sphericity”. Relationships between longitude, girth and ash dieback score were assessed using a correlation test. The statistical analyses were carried out using PASW Statistics 18™.

Results

Veteran ash trees affected by ash dieback are spread out over the whole county; no part was free of the disease. More veteran ash trees have been infected in 2015 compared with 2013, 2011 and 2009 (chi2$_{0.05;\ 12}$=131.27 $p<0.001$). There were more healthy trees in 2009, more affected trees in 2011 and more significantly affected and dead trees in 2013 and 2015 than would be expected according to a random distribution.

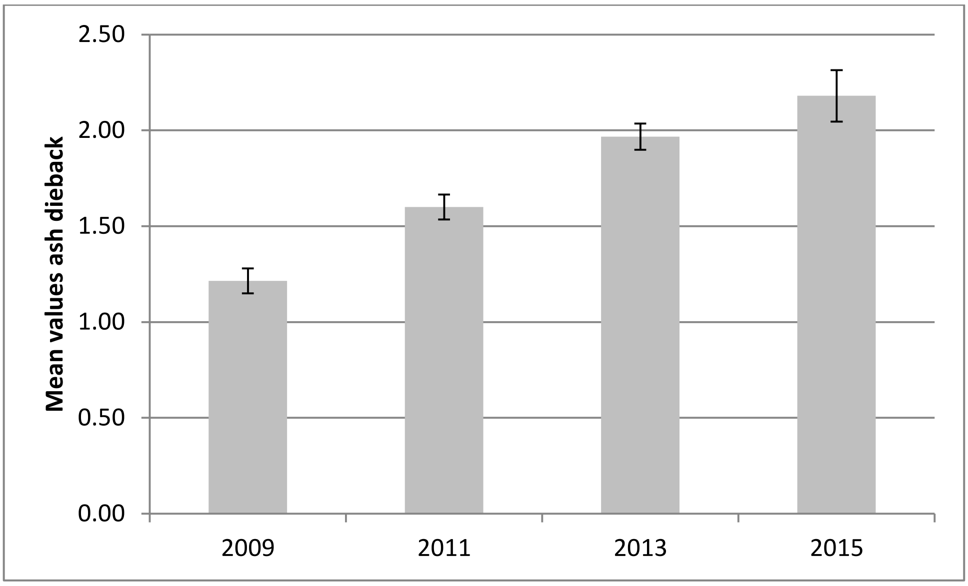


Figure 2 Mean values for ash dieback class have increased between the years where 0 is healthy, 1 is lightly affected, 2 is affected, 3 is significantly affected and 4 is dead.

90% of the veteran ash trees had some symptoms of ash dieback in 2015, compared with 84% in 2013, 77 % and 62% in 2011 and 2009 respectively (figure 2). The proportion of ash trees that had died since 2013 was 4.3%, which gives a mortality rate of 2.15% per year, which is virtually the same as between 2011 and 2013 when it was 2.1% per year (Bengtsson, 2014). In total, 35 veteran ash trees have died since the monitoring began, which is equivalent to almost 11% over six years or 1.8% per year.

It was hoped that the proportion of the population that would show symptoms would begin to flatten out (figure 3), given that ash dieback has been in Sweden since at least 2001 (Barklund, 2009).

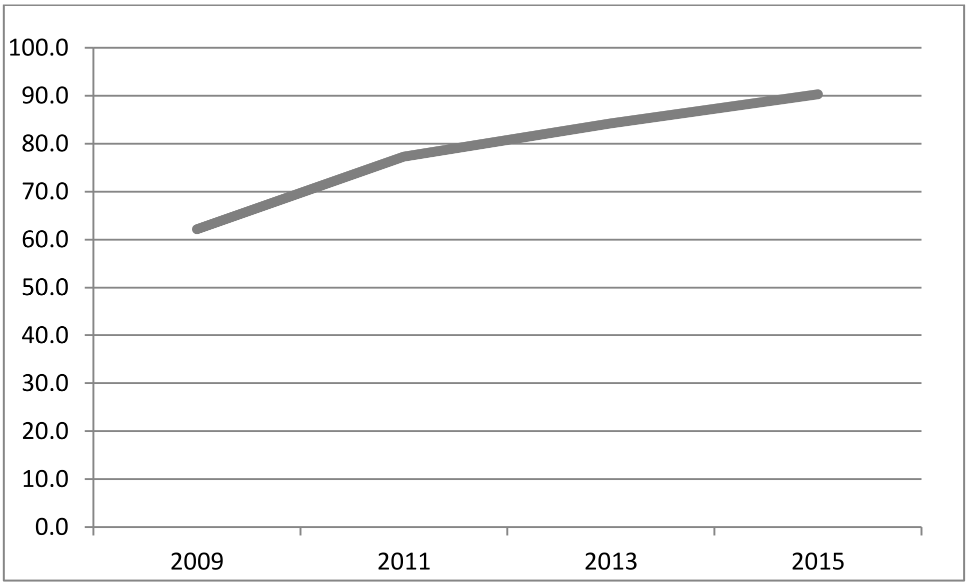


Figure 3 90% of the veteran ash trees had symptoms of ash dieback in 2015 compared with 84%, 77% and 63% in 2013, 2011 and 2009 respectively.

It was also interesting to note how the trees had moved between the classes over the six year period since the monitoring began (Figure 4). Many more veteran ash trees have developed symptoms and the most common change was that trees that were symptom free in 2009 are now lightly affected. However the progress can clearly also be rapid and worrying results are that 14 trees, which had no symptoms in 2009, were dead in 2015 and 22 trees that were healthy in 2009, were significantly affected in 2015.

In contrast the health of a number of trees was recorded as having improved which was an unexpected result. This is in line however, with other studies (Thomsen, 2010) which found that some trees produced lots of new shoots as a response to the disease and potentially resulting in the crown appearing to be in better condition than previously. It can also be explained by the fact that some of the trees in this study have been cut and thus the sick branches may have been removed. The last couple of years have been favourable for growth for ash (and thus perhaps the trees have been able to produce more leaves and new twigs) something that could explain a reduction in the amount of crown affected.

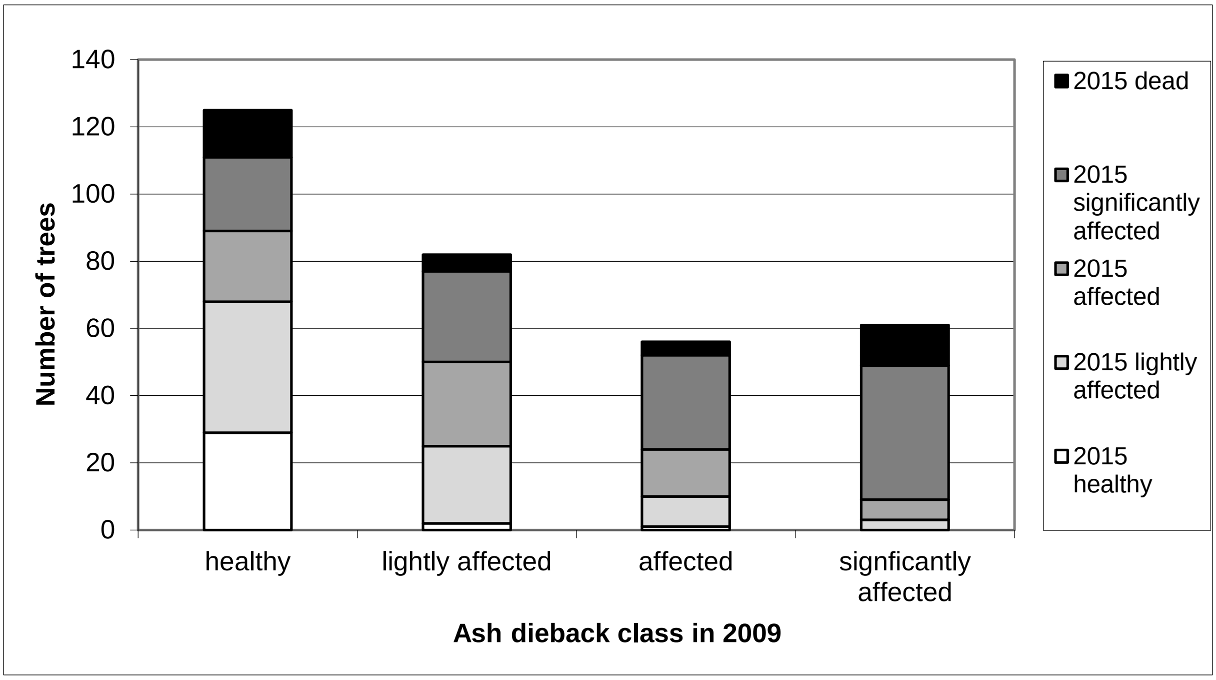


Figure 4 Movement between the ash dieback classes. The diagram shows the number of veteran ash trees in different ash dieback classes in 2015 divided up according to how badly affected they were in 2009.

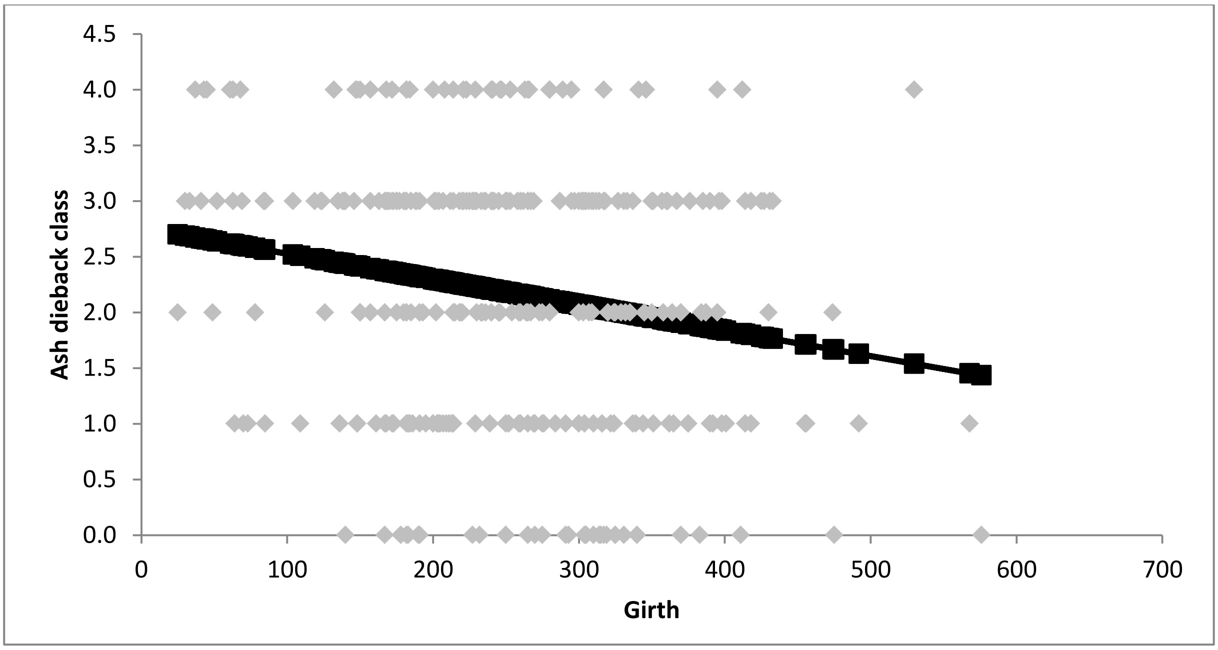


Figure 5 There is a trend that shows that ash trees with a larger girth are not as affected by ash dieback. The scale on the y-axis is ash dieback class where 0=healthy and 4=dead.

In 2009 there was no correlation between girth and ash dieback (Bengtsson & Stenström, 2009) but in 2013 the correlation between girth and the impact of ash dieback was statistically significant where larger ash trees were less affected (Bengtsson, 2014). In 2015 there was also a correlation (F=11.71; p=0.0007; r^2=0.034) that larger trees were less affected. Pollarded trees can have a smaller girth due to pollarding which slows the growth rates. To exclude pollarding as an explanatory factor, a similar analysis was carried out only with veteran ash trees that had not been pollarded. The result was not significant (F=2.38; p=0.125; r^2=0.021) and this is different from the result in 2013 (Bengtsson, 2014). The results do show however, that no ash tree under 140 cm in girth (equivalent to 45 cm diameter) was healthy in 2015. This trend explains only a small amount of the variation in the disease.

In 2013 data was also collected regarding the degree of openness and if the ash trees were in a grazed area. There were no differences in how badly affected the trees were in relation to how shaded they were (woodland, semi-open, open) or if the trees were in a grazed area or not (Bengtsson, 2014). In 2015 no statistical significance was found either (2-way ANOVA F=1.19, p= 0.315). This suggests that the environment in which the tree is standing plays a smaller part in explaining how badly affected the tree is.

Pollarding and Ash Dieback

The impact of ash dieback appears to some extent to be related to the management history of individual trees, but the relationship is complicated and varied between the years studied (Bengtsson *et al*, 2013, Bengtsson, 2014). In 2015, a repeated measures ANOVA test with pollarding as a factor was undertaken using data from four separate occasions (2009, 2011, 2013, 2015), following correction of the p-value with Huynh-Feldt correction for"sphericity". All years were significantly different from one another (Year: F=919.6, p<0.0001; figure 6). Trees which have never been pollarded are more affected by ash dieback than old lapsed pollards (pollards that have not been cut for more than 30 years) (F=3.94, p=0.02). Trees which have been pollarded more recently (ten years ago or less) were however not significantly different from non-pollarded trees (p= 0.222) or old lapsed pollards (p=0.627). This is a different result from previous years when both groups of pollarded trees were healthier than the maiden ash trees (Bengtsson, 2014).

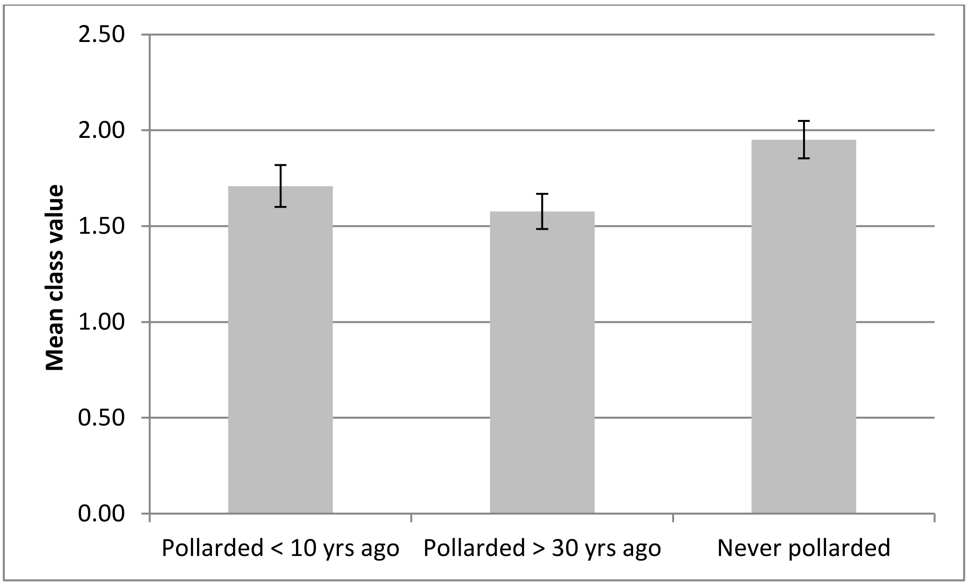


Figure 6 The impact of ash dieback between the different groups of trees studied where 0=healthy, 1=lightly affected, 2=affected, 3=significantly affected, 4=dead. Repeated measures ANOVA showed that trees pollarded more than 30 years ago, were healthier than trees that have never been pollarded (Year: F=919.6, p<0.0001).

Discussion

It is interesting that our study showed that trees with a larger girth were not as badly affected by ash dieback. The relationship between tree size and age, and the impact of dieback is not yet well-understood. Skovsgaard et al (2010) showed that the impact of ash dieback was greater on trees that were of smaller than average size which is consistent with the results from our study, but the reason why ash trees with a larger girth seem to have fewer symptoms is currently unclear. Larger girth may be connected to greater age and one could speculate that older trees have a different community of endophytes that influence the resistance of the tree or the pace of fungal spread. It could also be as simple as that it takes longer for the fungus to move through a larger tree and that the larger trees may have a more complex branch architecture.

As might be expected the veteran ash trees have become more affected over the years. In 2015, only 10% of the population were free of ash dieback symptoms. There appears to be little sign that the proportion of trees being affected is levelling out. According to several studies (Gross et al, 2015; Sandberg, 2015; Kjaer *et al*, 2013) there is only a very small proportion of the ash population that is tolerant or resistant to the fungus.

The annual mortality rate of the veteran ash trees in the county of Västra Götaland was 2.15% between 2013 and 2015. There is little data on the mortality rates of veteran ash trees with which to compare from before ash dieback, but other species of veteran trees (mainly beech and oak) have generally lower rates of mortality (authors own unpublished data, Read et al, 2010; Bengtsson and Fay, 2009). It is perhaps however, a slower rate of loss than might have been expected. A total of 11% (35 trees) have died from the entire population since 2009.

The results from this study with regard to pollarding are difficult to interpret because they varied between the survey years. It is interesting to note however that old lapsed pollards (not cut for more than 30 years) are showing less symptoms compared with maiden or uncut ash trees in the analysis that compared all years with pollarding as a factor. There was however no statistically significant difference between the group of ash trees that have been pollarded more recently in the analysis done in 2015. Maiden trees were however still the group most affected by ash dieback. The group of trees that have been pollarded more recently (in the last ten years) varies much more widely than either the lapsed pollards or maiden trees, as some of these trees are still in a regular cutting cycle in contrast to the other two groups. However, as the exact date of pollarding for each tree and the time at which the disease arrived at the tree's location are unknown, this may explain why the results have varied between years. It is possible that pollarding removes the affected shoots and thus the fungus, providing temporary respite from the disease. The recent pollards generally have a smaller crown and thus the proportion with symptoms may appear to be more when the new growth has become affected. More research is required in this area. It would be especially useful if pollarding could take place under controlled conditions or more data could be collected from old pollards to allow a more in depth analysis. This might help to gain a greater understanding of the role of pollarding in the pace of development of the disease.

Old pollards (both those in a regular cycle and lapsed) are interesting because they often have a more complex trunk and crown structure compared with maiden trees. Pollards exhibit more often the feature of having separate functional units, which are relatively independent of one another. This is a survival strategy where different parts of the same tree can to some extent behave like separate individuals. Each of these functional units can cater for their own energy requirements and take up water and nutrients from the closest part of the root system. Even if there may be a connection between these units, they can for example respond differently to the same type and amount of pruning (Lonsdale, 2013). This could be one explanation for why pollarded ash trees show fewer symptoms than maiden trees (pers. comm. Lonsdale, 2013) and may be an advantage due to the fact that the fungus has difficulties in moving through a more complex branch structure (Gross et al, 2014) and it can thus not as easily girdle the entire stem.

The variation within the groups of pollarded trees makes it difficult to make any detailed recommendations. It would seem sensible however to continue pollarding ash trees that are in a regular cutting cycle and which are healthy and even if they show symptoms of the disease as it may be possible to remove the fungus through cutting (pers. Comm. Stenlid). It would be advisable however to avoid pollarding all trees in the same year and to spread the cutting out (and thus any risks) over a longer time period (Bengtsson, 2016). With regard to lapsed pollards and maidens, it is important to note that all types of cutting on old trees are a stress for the tree. Cutting old ash trees can have significant consequences even if they are not affected by ash dieback (Lonsdale, 2013). Other studies (Eklund, 2009; Skovsgaard *et al*, 2010; Bakys *et al*, 2011, Skogsstyrelsen, 2013) have shown that old ash pollards that have cut hard in an attempt to restore them back into a pollarding cycle after a long period of lapse are weakened and thus may be more at risk from ash dieback. The authors therefore recommend *avoiding* all types of cutting on old lapsed and maiden ash trees if there is no acute risk of them falling apart. The other issue here is that there is the potential, through cutting, that the distance between the shoots, where infection primarily occurs, and the main stem is shortened, potentially speeding up the rate at which the fungus may enter the main stem of the tree (Stenlid, 2013).

Figure 7 A few old pollards which were last cut in 2012 and which show different levels of ash dieback. The tree in the middle was alive in 2013, but had died by 2015. The tree furthest away has no symptoms of ash dieback.

Figure 8 *Inonotus hispidus* – a fungus which is on the Red Data Book in Sweden and associated with ash.

There is currently no 'cure' for ash dieback. As the fungus seems to be spread by the wind it can spread a long way and there is little benefit in felling individual trees that are affected to reduce the risk of the disease spreading. Indeed there may even be a risk in removing trees as a preventative measure because just those trees could hold the key to the future conservation of ash and the associated species. The most important thing we can focus on now is to collect as much information as possible regarding ash trees which appear to have an increased resistance to the disease. It is also important to continue monitoring the development of the disease across the whole population. It may also be worth considering creating new pollards on young ash trees that are currently symptom-free and monitor their progress.

Bibliography

The original reports from which this article was compiled were written in 2009, 2012, 2014 and 2016; they are in Swedish, with English summaries and are available to download free from http://www.lansstyrelsen.se/vastragotaland/Sv/publikationer/Pages/default.aspx?year=&catSub=&doctype=1&freeText=askskottsjuka&numItems=

Barklund, P. (2009). *www-skogsskada.slu.se. Skadebeskrivning – Askskottsjuka.*

Bakys, R. Vasaitis, R., Barklund, P., Ihrmark, K. and Stenlid, J. (2009). *Investigations concerning the role of Chalara fraxinea in declining Fraxinus excelsior.* Plant Pathology 58, 284–292

Bakys, R. Vasaitis, R., Barklund, P., Thomsen, I.M. and Stenlid, J. (2009). *Occurrence and pathogenicity of fungi in necrotic and non-symptomatic shoots of declining common ash (Fraxinus excelsior) in Sweden*. Eur J Forest Res (2009) 128:51–60

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, 26:2, 128-135

Bakys R, Vasaitis R., Skovsgaard JP. (2013). *Patterns and Severity of Crown Dieback in Young Even-Aged Stands of European Ash (Fraxinus excelsior L.) in Relation to Stand Density, Bud Flushing Phenotype, and Season.* Plant Protection Science. 2013;49(3):120.

Baral, H., Queloz, V. & Hosoya, T. 2014, *Hymenoscyphus fraxineus, the correct scientific name for the fungus causing ash dieback in Europe*. IMA fungus, vol. 5, no. 1, pp. 79-80.

Bengtsson, S.B.K., Vasaitis, R., Kirisits, T., Solheim, H. and Stenlid, J. (2012). *Population structure of Hymenoscyphus fraxinus and its genetic relationship to Hymenoscyphus albidus.* Fungal Ecol. 5, 147–153.

Bengtsson, S.B.K., Barklund P., Brömssen C. V., Stenlid J.(2014). *Seasonal Pattern of Lesion Development in Diseased Fraxinus excelsior Infected by Hymenoscyphus pseudoalbidus*: e76429. PLoS One. 2014;9(4).

Bengtsson, V & Stenström, A. (2009). *Inventering av askskottsjuka i Västra Götalands län 2009. Länsstyrelsen i Västra Götalands län*, Naturvårdsenheten, rapport 2009:80.

Bengtsson, V, Stenström, A & Finsberg, C. (2012). *Askskottsjuka –ett nytt hot mot våra skyddsvärda träd?*(Ash dieback – a new threat to our veteran trees? English summary), Naturvårdsenheten, rapport 2012:29.

Bengtsson, V, Stenström, A & Finsberg, C. (2013). *The impact of ash dieback on veteran and pollarded trees in Sweden*. Quarterly J. Forestry, 107 (1), 27-33.

Bengtsson, V. (2014) *Askskottsjuka –ett fortsatt hot mot våra skyddsvärda träd?* (Ash dieback – a continuing threat to our veteran trees? English summary), Naturvårdsenheten, rapport 2014:17.

Bengtsson, V. (2016) *Askskottsjuka –hur mår våra skyddsvärda ask träd?* (Ash dieback – further monitoring of our veteran trees)(English summary), Naturvårdsenheten, rapport 2016:xx (in draft).

Cleary, M.R., Arhipova, N., Gaitnieks, T., Stenlid, J. and Vasaitis, R. (2012). *Natural infection of Fraxinus excelsior seeds by Chalara fraxinea*. For. Pathol. 43, 83–85.

Cleary MR, Andersson PF, Broberg A, Elfstrand M, Daniel G, Stenlid J. (2014). *Genotypes of Fraxinus excelsior with different susceptibility to the ash dieback pathogen Hymenoscyphus pseudoalbidus and their response to the phytotoxin viridiol - a metabolomic and microscopic study.* Phytochemistry. 2014;102:115.

Eklund, S. (2009). *Hamling av Ask, Fraxinus excelsior, och hur det påverkar trädets utsatthet för askskottsjukan*. Examensarbete Skövde Högskola.

Ellis, C.J., Coppins, B.J., Eaton, S. & Simkin, J. (2013). *Implications of Ash Dieback for Associated Epiphytes.* Letters: Conservation Biology, Volume 27, No. 5, 898–901

Enderle R, Peters F, Nakou A, 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. 2013;132(5):865-76.

Enderle R, Nakou A, Thomas K, Metzler B. (2015). *Susceptibility of autochthonous German Fraxinus excelsior clones to Hymenoscyphus pseudoalbidus is genetically determined.* Annals of Forest Science. 2015;72(2):183-93.

EPPO, (2010). http://archives.eppo.org/MEETINGS/2010_conferences/chalara_oslo.htm

FRAXBACK, (2013a). *Frontiers in Ash Dieback research*, Malmö, September 2013.

FRAXBACK, (2013b). *Living with ash dieback in continental Europe: present situation, long-term experience, and future perspectives* London, 2013. http://www.youtube.com/channel/UCI810ZkJIgiS9ALeeT5tq3g ?feature=watch

Gaitnieks, T., Arhipova, N., Ruņģis, D., Laiviņš M. & Vasaitis R. (2013). *Common ash (Fraxinus excelsior) dieback in Latvia: natural regeneration of declining ash stands.* FRAXBACK 2013b, London.

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V and Sieber, T.N. (2013). *Hymenoscyphus fraxinus, the causal agent of European ash dieback.* Molecular Plant Pathology. DOI: 10.1111/mpp.12073

Gross, A. and Holdenrieder, O. (2013) *On the longevity of Hymenoscyphus fraxinus in petioles of Fraxinus excelsior*. For. Pathol. 43, 168–170.

Gross, A., Zaffarano, P.L., Duo, A. and Grünig, C.R. (2012) *Reproductive mode and life cycle of the ash dieback pathogen Hymenoscyphus fraxinus*. Fungal Genet.Biol. 49, 977–986.

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V. and Sieber, T. N. (2014), *Hymenoscyphus pseudoalbidus, the causal agent of European ash dieback.* Molecular Plant Pathology, 15: 5–21. doi: 10.1111/mpp.12073

Gross A, Hosoya T, Queloz V. (2014) *Population structure of the invasive forest pathogen Hymenoscyphus pseudoalbidus.* Molecular Ecology. 23(12):2943-60

Gross, A., Holdenrieder, O. (2015), *Pathogenicity of Hymenoscyphus fraxineus and Hymenoscyphus albidus towards Fraxinus mandshurica var. japonica.* Forest Pathology, 45: 172–174. doi: 10.1111/efp.12182

Hauptman, T., Celar, F., de Groot, M. & Jurc, D. (2015). *Application of fungicides and urea for control of ash dieback*, Forest - Biogeosciences and Forestry,vol. 8, no. 2, pp. 165-171.

Hietala, A.M., Timmermann, V. and Børja, I. (2013) *The invasive ash dieback pathogen Hymenoscyphus fraxinus exerts maximal infection pressure prior to the onset of host leaf senescence.* Fungal Ecol. 6, 302–308.

Husson, C., Caël, O., Grandjean, J.P., Nageleisen, L.M. and Marçais, B. (2012). *Occurrence of Hymenoscyphus fraxinus on infected ash logs*. Plant Pathol. 61,889–895.

Jönsson, M.T. and Thor, G. (2012) *Estimating coextinction risks from epidemic tree death: affiliate lichen communities among diseased host tree populations of Fraxinus excelsior*. PLoS ONE, 7, e45701.

Junker, C., Mandey, F., Pais, A., Ebel, R. and Schulz, B. (2013) *Hymenoscyphus fraxinus and Hymenoscyphus albidus: viridiol concentration and virulence do not correlate*. For. Pathol. (in press). doi:10.1111/efp.12066

Kirisits, T. and Freinschlag, C. (2012) *Ash dieback caused by Hymenoscyphus fraxinus in a seed plantation of Fraxinus excelsior in Austria.* J. Agric. Ext. Rural Dev. 4, 184–191.

Kirisits T, Woodward S.(2015). *Ascocarp formation of Hymenoscyphus fraxineus on several year old pseudosclerotial leaf rachises of Fraxinus excelsior*. Forest Pathology. 2015;45(3):254-7.

Kjær, E.D., McKinney, L.V., Nielsen, L.R., Hansen, L.N. and Hansen, J.K. (2012). *Adaptive potential of ash (Fraxinus excelsior) populations against the novel emerging pathogen Hymenoscyphus fraxinus.* Evol. Appl. 5, 219–228.

Kowalski, T. 2006. *Chalara fraxinea sp. nov. associated with dieback of ash (Fraxinus excelsior) in Poland*. Forest Pathology 36:264–270.

Kowalski, T. and Holdenrieder, O. (2009)a. *The teleomorph of Chalara fraxinea, the causal agent of ash dieback.* For. Path. 39 (2009) 304–308.

Kowalski, T. and Holdenrieder, O. (2009)b, *Pathogenicity of Chalara fraxinea. Forest Pathology*, 39: 1–7. doi: 10.1111/j.1439-0329.2008.00565.x

Littlewood NA, Nau BS, Pozsgai G, Stockan JA, Stubbs A, Young MR. Invertebrate species at risk from Ash Dieback in the UK. Journal of Insect Conservation. 2014;2015;19(1):75.

Albin Lobo, A,. Hansen J.K., McKinney L.V., Nielsen L.R. & Kjær E.D. (2014) *Genetic variation in dieback resistance: growth and survival of Fraxinus excelsior under the influence of Hymenoscyphus pseudoalbidus,* Scandinavian Journal of Forest Research, 29:6, 519-526, DOI: 10.1080/02827581.2014.950603

Lobo A., McKinney L.V., Hansen J.K., Kjær E.D., Nielsen L.R., Holdenrieder O. (2015). *Genetic variation in dieback resistance in Fraxinus excelsior confirmed by progeny inoculation assay*. Forest Pathology. 2015;45(5):379-87.

Lõhmus, A. & Runnel, K. (2014). *Ash dieback can rapidly eradicate isolated epiphyte populations in production forests: A case study,* Biological Conservation,vol. 169, pp. 185-188.

Lonsdale, (ed), 2013. *Ancient and other veteran trees: further guidance on management*. The Tree Council, London.

Lonsdale, D. (2013). *The recognition of functional units as an aid to tree management with particular reference to veteran trees*. Arboricultural Journal, 2013 Vol 35, No 4, pp 188-201.

Lygis, V., Bakys R., Gustienė A., Burokienė D., Matelis A. & Vasaitis R. (2013) *Lithuania: forest regeneration in ash dieback-affected sites subjected to sanitary fellings.* FRAXBACK 2013b, London.

Lygis V, Bakys R, Gustiene A, Burokiene D, Matelis A, Vasaitis R. (2014). *Forest self-regeneration following clear-felling of dieback-affected Fraxinus excelsior: focus on ash.* European Journal of Forest Research. 2014;133(3):501-10.

McKinney L V, Nielsen L R, Hansen J K and Kjær E D (2011). *Presence of natural genetic resistance in Fraxinus excelsior (Oleraceae) to Chalara fraxinea (Ascomycota): an emerging infectious disease.* Heredity 106, 788–797; doi:10.1038/hdy.2010.119

McKinney, L.V., Thomsen, I.M., Kjær, E.D., Bengtsson, S.B.K. and Nielsen, L.R. (2012a) *Rapid invasion by an aggressive pathogenic fungus (Hymenoscyphus fraxinus) replaces a native decomposer (Hymenoscyphus albidus): a case oflocal cryptic extinction?* Fungal Ecol. 5, 663–669.

McKinney, L. V., Thomsen, I. M., Kjær, E. D. and Nielsen, L. R. (2012b), *Genetic resistance to Hymenoscyphus fraxinus limits fungal growth and symptom occurrence in Fraxinus excelsior*. Forest Pathology. 42, 69-74. doi: 10.1111/j.1439-0329.2011.00725.x

McKinney, L.V., Nielsen, L.R., Collinge, D.B., Thomsen, I.M., Hansen, J.K. & Kjær, E.D. (2014). *The ash dieback crisis: genetic variation in resistance can prove a long term solution*, Plant Pathology, vol. 63, no. 3, pp. 485-499

Mitchell, R.J., Beaton, J.K., Bellamy, P.E., Broome, A., Chetcuti, J., Eaton, S., Ellis, C.J., Gimona, A., Harmer, R., Hester, A.J., Hewison, R.L., Hodgetts, N.G., Iason, G.R., Kerr, G., Littlewood, N.A., Newey, S., Potts, J.M., Pozsgai, G., Ray, D., Sim, D.A., Stockan, J.A., Taylor, A.F.S. & Woodward, S.(2014). *Ash dieback in the UK: A review of the ecological and conservation implications and potential management options*,Biological Conservation,vol. 175, pp. 95-109.

Orlikowski, L. B., Ptaszek, M., Rodziewicz, A., Nechwatal, J., Thinggaard, K. and Jung, T. (2011), *Phytophthora root and collar rot of mature Fraxinus excelsior in forest stands in Poland and Denmark*. Forest Pathology. doi: 10.1111/j.1439-0329.2011.00714.x

Pautasso, M., Dehnen-Schmutz, K., Holdenrieder, O., Pietravalle, S., Salama, N., Jeger, M.J., Lange, E. and Hehl-Lange, S. (2010) *Plant health and global change—some implications for landscape management.* Biol. Rev. 85, 729–755.

Pautasso, M., Thomas F. Döring, T.F., Garbelotto M., Pellis L. & Jeger M.J. (2012). *Impacts of climate change on plant diseases—opinions and trends.* Eur J Plant Pathol (2012) 133:295–313 DOI 10.1007/s10658-012-9936-1

Pautasso, M., Aas, G., Queloz, V. and Holdenrieder, O. (2013) *European ash (Fraxinus excelsior) dieback—a conservation biology challenge*. Biol. Conserv. 158, 37–49.

Pautasso, M., Schlegel, M. & Holdenrieder, O. (2015). *Forest Health in a Changing World,* Microbial Ecology,vol. 69, no. 4, pp. 826-842.

Przybył, K. (2002) *Fungi associated with necrotic apical parts of Fraxinus excelsior shoots.* For. Pathol. 32, 387–394.

Queloz, V., Grünig, C. R., Berndt, R., Kowalski, T., Sieber, T. N. and Holdenrieder, O. (2011), *Cryptic speciation in Hymenoscyphus albidus*. Forest Pathology, 41: 133–142. doi: 10.1111/j.1439-0329.2010.00645.x

Read, H. (2000). *Veteran trees: A guide to good management*. Peterborough: English Nature.

Roberge, J.M., Bengtsson, S.B.K., Wulff, S. & Snäll, T. (2011). *Edge creation and tree dieback influence the patch-tracking metapopulation dynamics of a red-listed epiphytic bryophyte*. Journal of Applied Ecology, 48(3), pp. 650-658.

Sandström, J., Bjelke, U., Carlberg, T. & Sundberg, S. (2015*). Tillstånd och trender för arter och deras livsmiljöer- rödlistade arter i Sverige 2015*. (Artdatabanken Rapporterar, 17). SLU, Uppsala.

Schoebel CN, Zoller S, Rigling D. (2014). *Detection and genetic characterisation of a novel mycovirus in Hymenoscyphus fraxineus, the causal agent of ash dieback. Infection, genetics and evolution* : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases. 2014;28:78-86.

Schumacher, J (2010) http://archives.eppo.org/MEETINGS/2010_conferences/chalara/14_Schumacher/index.html

Schumacher, J., Kehr, R. and Leonhard, S. (2010), *Mycological and histological investigations of Fraxinus excelsior nursery saplings naturally infected by Chalara fraxinea*. Forest Pathology, 40: 419–429. doi: 10.1111/j.1439-0329.2009.00615.x

Sieber TN (2007) *Endophytic fungi in forest trees: are they mutualists?* Fungal Biol Rev 21:75–89. doi:10.1016/j.fbr.2007.05.004

Skogsstyrelsen (2013). *Ask och askskottsjukan i Sverige.* (Meddelande 4). Jönköping.

Skogsdata 2012. *Riksskogstaxeringen*, SLU. Umeå.

Skovsgaard, J. P., Thomsen, I. M. & Barklund, (2009). *Skötsel av bestånd med askottsjuka (Management of stands with ash dieback in Swedish only).* Faktaskog 13 2009.

Skovsgaard, J. P., Thomsen, I. M., Skovgaard, I. M. and Martinussen, T. (2010), *Associations among symptoms of dieback in even-aged stands of ash (Fraxinus excelsior L.).* Forest Pathology, 40: 7–18. doi: 10.1111/j.1439-0329.2009.00599.x

SLU, 2010. Rödlistade arter i Sverige (Sweden’s Red Data Book). SLU, 2010. http://www.artfakta.se/GetSpecies.aspx?SearchType=Advanced

Stener, L.-G. (2012) *Clonal differences in susceptibility to the dieback of Fraxinus excelsior in southern Sweden*. Scand. J. For. Res. 28, 205–216.

Stenlid, J. (2013). *Askskottsjukan visar riskerna med global handel. In* Björkman, C. & Stenlid, J. (red.) (2013). Svampar och insekter Rapport från Future Forests 2009-2012. Future Forests rapport series 2013:5. Sveriges lantbruksuniversitet, Umeå, 44 pp.

Strandberg, S. (2015). Ash decline in Jönköping and Östergötland counties:-current status and future prospects for Fraxinus excelsior. Master thesis, Uppsala, SLU. http://stud.epsilon.slu.se.

Tellenbach C, Sieber TN (2012). *Do colonization by dark septate endophytes and elevated temperature affect pathogenicity of oomycetes?* FEMS Microbiol Ecol 82:157–168

Tellenbach C, Sumarah MW, Grünig CR, Miller JD (2013). *Inhibition of Phytophthora species by secondary metabolites produced by the dark septate endophyte Phialocephala europaea.* Fung Ecol 6:12–18. doi:10.1016/j.funeco.2012.10.003

Thomsen, I. M (2010). Impact of Chalara fraxinea on Danish forests. http://archives.eppo.org/MEETINGS/2010_conferences/chalara/08_Thomsen/index.html

Witzell J, Martín JA, Blumenstein K (2014) *Ecological aspects of endophyte-based biocontrol of forest diseases.* In: Verma VC, Gange AC (eds) Advances in endophytic research. Springer, Berlin, pp 321–333. doi:10.1007/978-81-322-1575-2_17

Zhao, Y.-J., Hosoya, T., Baral, H.-O., Hosaka, K. and Kakishima, M. (2012) *Hymenoscyphus fraxinus, the correct name for Lambertella albida reported from Japan.* Mycotaxon, 122, 25–41.

Biography of authors

Vikki Bengtsson is the corresponding author at Pro Natura vikki.bengtsson@pro-natura.net and has worked professionally with nature conservation since 1992 both in the UK and in Sweden. Vikki has primarily worked with issues relating to the practical management and restoration of ancient trees and now works as a freelance ecologist in Sweden with Pro Natura. Vikki is also a trustee of the Ancient Tree Forum.

Anna Stenström has a PhD in plant ecology. Anna works in the Nature Conservation Department at the County Administrative Board of Västra Götaland in western Sweden. Anna is responsible for implementation of the Action Plan for Veteran Trees in the County of Västra Götaland, which includes surveying, restoration and monitoring activities. She is also responsible for the implementation of an additional 25 action plans for threatened species in Västra Götaland.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and
Guidelines for Sustainable Management, 273 – 283.

An overview of ash species in Greece: ecology, biology and taxonomy, silviculture, genetics and health status

K. SPANOS*, D. GAITANIS

Hellenic Agricultural Organisation, General Directorate of Agricultural Research, Forest Research Institute
57006 -Thessaloniki, Greece
** Corresponding author: kspanos@fri.gr*

Abstract
Three Ash species are found in Greece: 1) *Fraxinus ornus* L., 2) *Fraxinus angustifolia* Vahl., and 3) *Fraxinus excelsior* L.. In this work an overview of the three ash species in Greece is presented and summarised as following: taxonomy, biology and ecology, silviculture and genetics of the three species. Summarised results on molecular genetics, provenance trials and propagation by stem cuttings of *Fraxinus angustifolia* are also presented. The general health status of ash is also discussed and analysed. No records of ash dieback (*Hymenoscyphus fraxineus*) infection have reported up to now. Furthermore, Conservation measures and strategy for protecting ash genetic resources in Greece are presented and recommedetions are cited.

Keywords: *Fraxinus* spp., taxonomy, ecology, biology, silviculture, genetics, health status, conservation.

1. Introduction

Native ash trees are highly valuable for their wood and non-wood products. Wood products include all products based on wood (e.g. lumber, paper, charcoal) whereas non-wood products are all other forest products not based on wood (e.g. nuts, seeds, fruits, bark, fodder, honey) including ecosystem services (e.g. biodiversity, water balance, aesthetics) (Calama et al., 2010).

Three Ash species are found in Greece (Spanos et al., 2004; Fraxigen 2005): 1) *Fraxinus ornus* - commonly found everywhere in the country in hilly and mountainous areas, 2) *F. angustifolia* - an almost threatened species in the lowlands – along the rivers and in wetlands, and 3) *Fraxinus excelsior* – a rare species in the mountains of Northern Greece in the borders with other Balkan countries (Bulgaria, FYR - Macedonia, Albania).

F. ornus is an ecologically important species and also widely used for ornamental purposes. It is mainly an insect pollinated species and has male and female flowers on different trees (Wallander 2001, Fraxigen 2005, Verdu et al. 2007). Locally it is used for making hand tools and may support the local economy.

F. angustifolia is mainly a wind pollinated species (but not excluding insect pollination to some extent) and has hermaphrodite flowers (Wallander 2001, Fraxigen 2005, Papi et al., 2012). It is a fast growing species and the mean annual increment on plantations on good sites can reach 20-25 m^3 ha^{-1} (Fraxigen 2005). In Greece, *F. angustifolia* is the most important ash species for its ecological restoration value in the lowlands - mainly in wetlands and river banks. Some remnant native stands (lowland wetlands) of this species have high conservation value and have been declared as Natural Monuments and also registered in the NATURA 2000 network (e.g. GR2310001) (Dafis et al., 2000; NATURA 2000). *F. excelsior* (Common ash) is mainly a wind pollinated species and may have male, female or hermaphrodite flowers on the same or different trees (Wallander 2001, Fraxigen 2005). *F. excelsior* (Common ash) is not an important tree species in Greece due to its rarity.

Ash dieback caused by *Hymenoscyphus fraxineus* (T. Kowalski) Baral et al. (=*Chalara fraxineua* T. Kowalski) is an emerging disease causing massive tree mortality threatening ash species (mainly *F. excelsior* and *F. angustifolia*) in Europe (Kirisits et al. 2009, Bakys 2013). Starting in early to mid-1990s, large-scale decline of *F. excelsior* was detected and observed in Lithuania and Poland (Kowalski 2006, Bakys 2013). Subsequently, the dieback of *F. excelsior* spread towards south, west and northern Europe, and has reported in more than 20 countries (Kirisits et al. 2009, Bakys 2013). However, despite of this serious threat in Europe, no data and information of this disease have been reported for the three native ash species in Greece.

In this work, an overview of the general status of the three ash species in Greece is presented and summarised.

2. Taxonomy, ecology, biology and silviculture of ash species in Greece

Fraxinus ornus

Its geographical distribution is shown in Figure 1. *F. ornus* L. is a small tree with white flowers in conspicuous inflorescences. The flowers are insect pollinated without excluding wind pollination (Dommee et al. 1999; Fraxigen 2005; Verdu et al. 2007). It is an ecologically important species and also widely used for ornamental purposes. It is mainly found on hilly and mountainous areas. In the past, it was used to be managed by the coppicing system (for fuelwood and fencing poles) but at present it is managed by the conversion system (coppicing and seed regeneration). Apart from occasionally damage by bacteria (e.g. *Pseudomonas* spp.) and defoliating insects (e.g. *Lymantria dispar*), no serious health problems for this species have been reported.

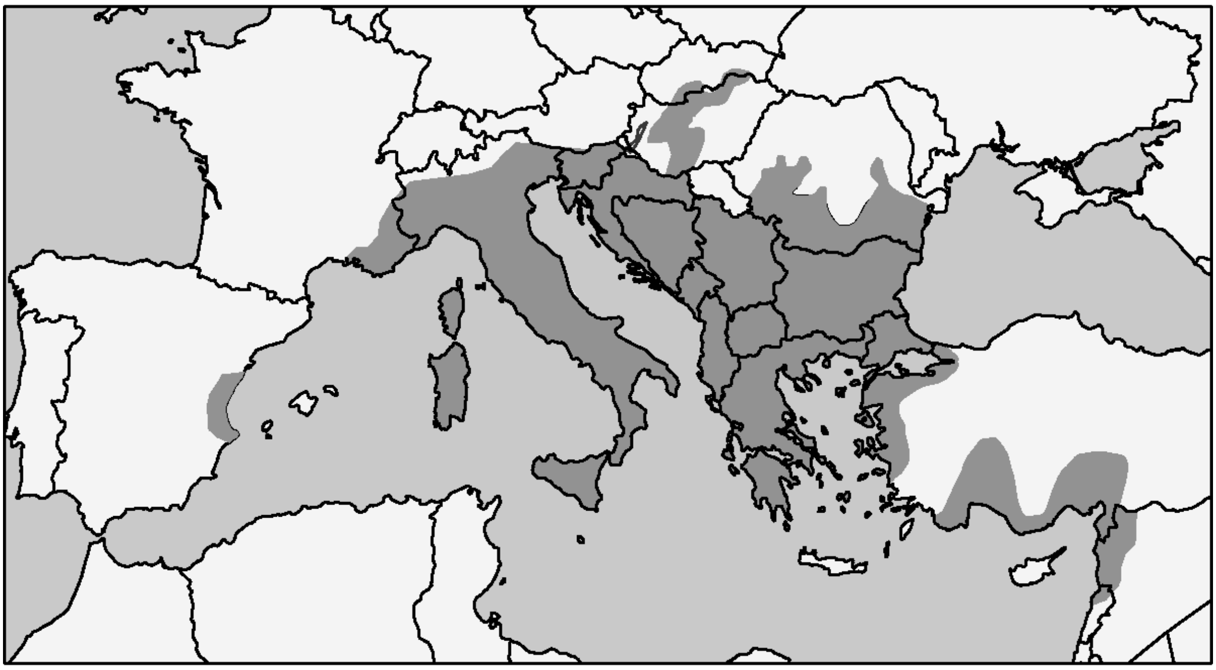


Figure 1 Geographic distribution of *Fraxinus ornus* in Greece, Europe and Asia (Fraxigen, 2005)

Fraxinus angustifolia

The tree species *F. angustifolia* Vahl. is one of the three native ash species in Europe (*Fraxinus excelsior*, *F. angustifolia*, *F. ornus* - Oleaceae) (Wallander 2001, Fraxigen 2005, Spanos et al. 2004, Verdu et al. 2007). Its geographical distribution (Europe, N. Africa and Asia) is shown in Figure 2. In Greece, it is mainly found in the following vegetation zones (alliances): QUERCION ILLICIS, OSTRYO-CARPINION and QUERCION FRAINETTO, pure or mixed with elm, poplar, oak and other broadleaved species along the rivers and in wetlands (Spanos et al. 2004). Some riparian or wetland forests of *F. angustifolia* - mixed with other noble hardwoods (e.g. *Ulmus* spp., *Quercus robur*, *Populus alba*, *Platanus orientalis, Alnus glutinosa*) - are considered of high biodiversity value and have been included in the NATURA 2000 network (FRAXIGEN 2005; Dafis et al., 2000).

It forms even-aged stands along the river banks and wetlands mainly in the lowlands.
It is a light demanding species.
It prefers humid rich and deep soils.
It is regenerated by seeds and stump and root sprouts.

Several subspecies, varieties or species have been reported for *F. angustifolia* but all proved to belong to the cluster species *Fraxinus angustifolia* (Fraxigen 2005). It is a diploid species ($2n$=46) and presents high intra-population genetic variation but low inter-population (Fraxigen, 2005; Papi et al., 2012). It is considered a fast growing noble hardwood and it is an interesting and important tree species for the country (multiple use and valuable timber). Its wood is relatively heavy (700-750 kg/m^3) and it has high calorific value (fuel wood). The wood is valuable with prices >800 EURO/m^3 (sawn timber). The wood properties are more or less similar to that of *F. angustifolia* (Fraxigen 2005; Spanos and Gaitanis 2015). The species is also important for woody biomass production and for green biomass (fodder) too. It has high coppicing and pollarding ability (Cicek and Yilmaz 2002, Fraxigen 2005, Spanos and Gaitanis 2015). It is an ideal tree for restoration and improvement of the riparian ecosystems and other wetlands as well as for temporary flooding lands (Fraxigen 2005, Spanos et al. 2004, Spanos and Gaitanis 2015). Furthermore, the species has been included in the official list of forest tree species used for afforestation of agricultural and abandoned lands (with potential subsidies) (Spanos and Gaitanis 2015). Nowadays, its natural stands are managed by the conversion system (coppicing and seed regeneration). The natural distribution of the species in Europe, Asia and N. Africa is presented in Figure 2.

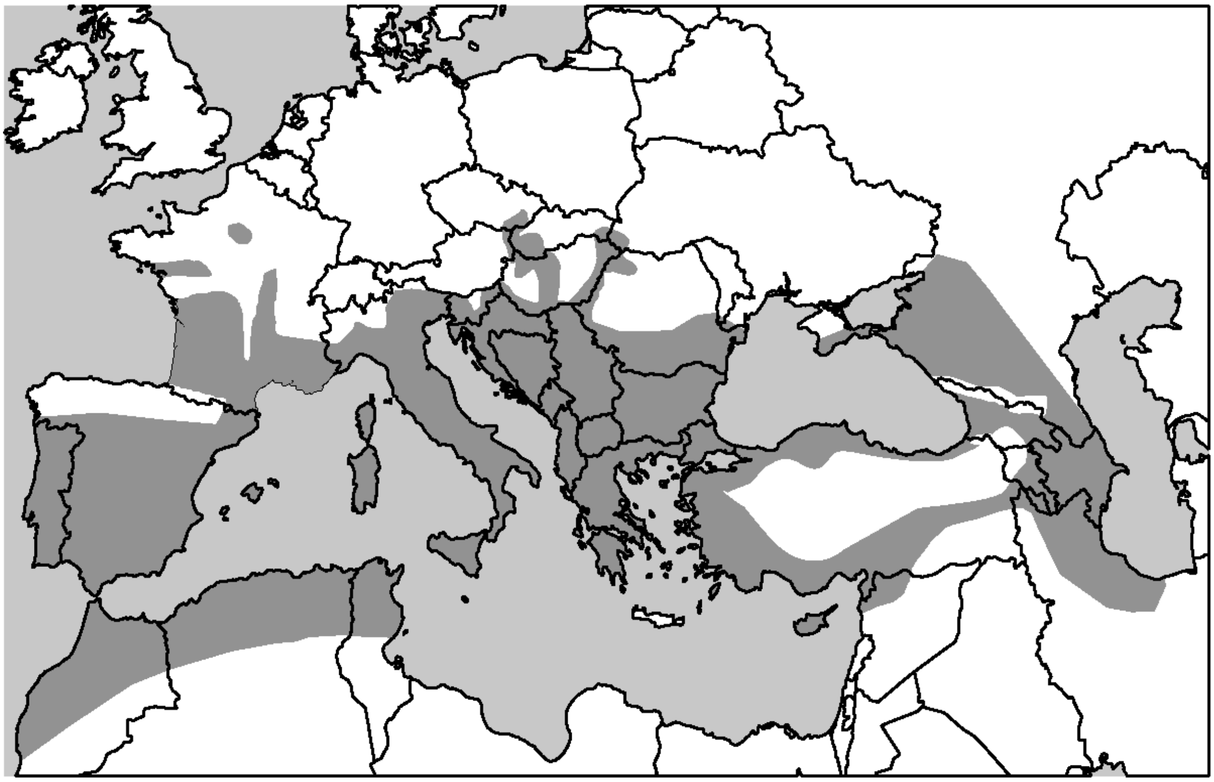


Figure. 2 Geographic distribution of *Fraxinus angustifolia* in Greece, Europe, Asia and N. Africa (Fraxigen, 2005).

Fraxinus excelsior

Its geographical distribution (Europe and Asia) is shown in Figure 3. It is widely distributed in Europe, but it is very rare in Greece. It is rarely found in individuals or in small groups of trees on the high mountains and also watersheds in N. Greece, in the borders with Albania, FYR – Macedonia and Bulgaria (in oak forests, beech or mixed beech-fir forests). Possible hybridization of the species with *F angustifolia* may happen where there is an overlap of their distributions (e.g. in Romania, Fraxigen 2005). It seems an interesting topic for a new research.

3. Genetic variation of *Fraxinus angustifolia* studied with DNA markers

A study on genetic variation of *Fraxinus angustifolia* was carried out in the year 2003 (Papi et al. 2003, Papi et al. 2012). For this study, eight (8) native populations of *F. angustifolia* were identified and selected all over continental Greece. Their haplotype diversity, total gene diversity, heterozygosity levels within populations and genetic diversity within and among populations were estimated.

Chloroplast DNA diversity was determined by 5 haplotypes. Haplotype H1 was the most dominant in Greece and in the Balkan Peninsula. The next most dominant was haplotype H3 and seems to have migrated northwest in Mediterranean area (Papi et al. 2012). Haplotype H5 was also found in one population. Although most of the studied populations showed a single haplotype, the majority of the detected haplotypes are found also in other populations from the Balkans suggesting that Greece could be the origin of most European cpDNA haplotypes (Heuertz et al. 2004; Papi et al. 2012).

Selected nuclear DNA markers coded as FEMSATL4, FEMSATL11, FEMSATL16, FEMSATL19 and M2-30 of nuclear microsatellite DNA were used to study genetic diversity. They revealed high total gene diversity within populations with relatively lower levels detected in the populations 'Evia' and 'Louros'. High polymorphism within populations and high total gene diversity (H_T = 0.784) were recorded for all molecular markers (Papi et al. 2012). Molecular markers FEMSATL4, FEMSATL11 and M2-30 were more polymorphic in comparison to FEMSATL16 and FEMSATL19. The mean total heterozygosity (for all populations) was found high for the molecular markers FEMSATL4, FEMSATL11 and M2-30 (Ho=0.703 – 0.909), relatively lower for the markers FEMSATL16 and FEMSATL19 (Ho=0.492-0.385), while the total mean was Ho=0.669. The mean observed heterozygosity (Ho) for all populations and all nuclear markers was relatively lower than the mean expected heterozygosity (Hs). Genetic differentiation among populations was found low (FST 0.024 – 0.133, total mean FST = 0.059), fact which explains only a small proportion of the total genetic diversity (Papi et al. 2012). Interestingly, 3 populations ('Evia', 'Komotini' and 'Louros') showed higher gene differentiation in comparison to the rest of the populations.

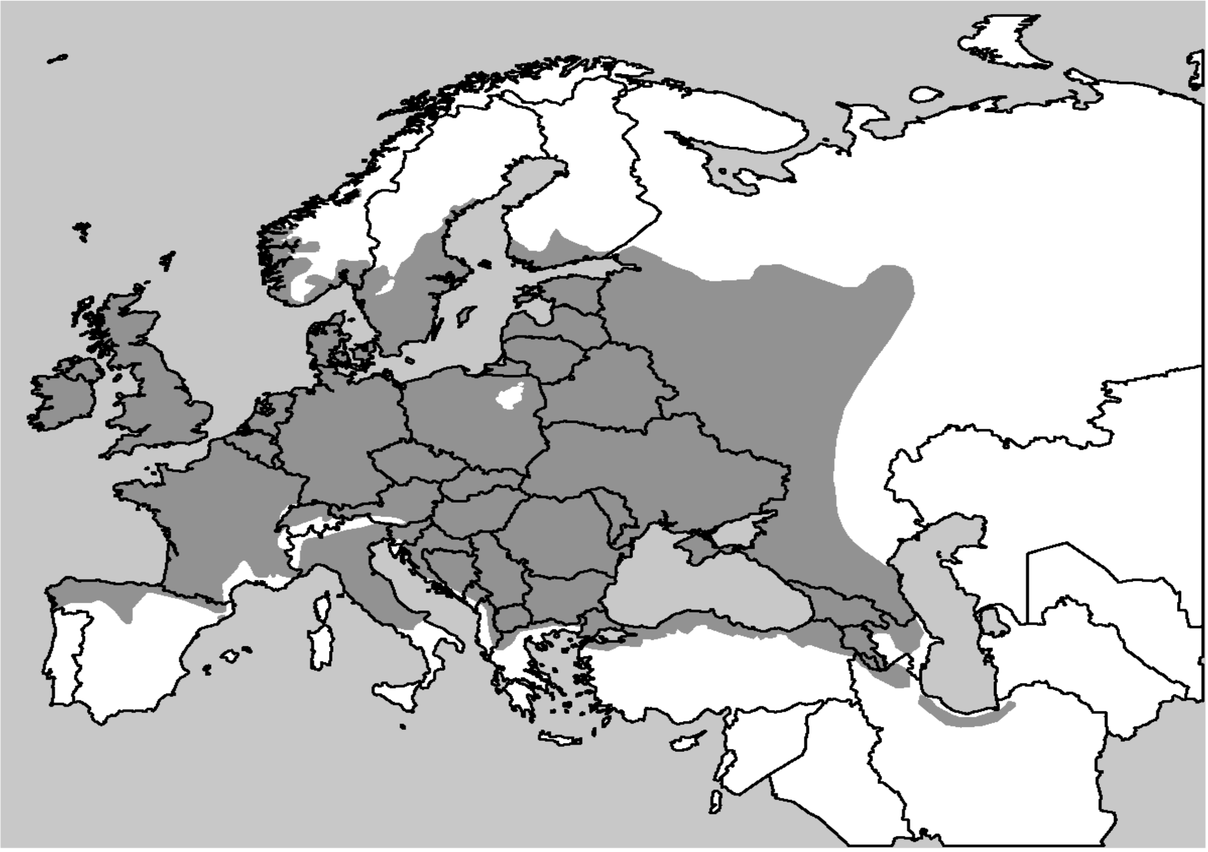


Figure 3 Geographic distribution of *Fraxinus excelsior* in Greece, Europe and Asia (Fraxigen 2005).

4. Ash provenance trials in Greece

Provenance trials of the native forest tree species *Fraxinus angustifolia* have been tested in Greece (FRI, Thessaloniki) (Spanos and Gaitanis, 2015). In the framework of the European research program FRAXIGEN (EVK2-CT-2001-00180), eight (8) natural populations (coded as: 08FAN, 09FAN, 11FAN, 12FAN, 13FAN, 14FAN, 15FAN, 16FAN) were identified all over continental Greece and selected for research (Table 1). Seeds were collected (30 trees/provenance in November, 2002) from the populations and 2 year-old nursery plants were developed. The aim of this trial was to compare eight (8) provenances of the species in relation to main biometric characteristics (DBH, total height). In December 2004, an experimental planting of provenance trial was established at the arboretum of the Forest Research Institute. Provenances were repeated (in a randomised block design) in 5 blocks with 15 trees per provenance. The planting space was 2 x 2 m. In December 2010, biometric data (height and diameter/DBH) were recorded and data analysis was done (Table 2 & 3). The average survival rate was 78% and the range between 69 and 87%. In relation to diameter, analysis showed significant differences ($P<0.001$) among provenances, significant differences ($p<0.01$) among blocks as well as of the interaction ($P<0.01$) of blocks with provenances. At this stage of the recording, almost all provenances depicted significant diameter growth. In relation to the total height, results did not show significant differences ($P>0.05$) among provenances, but significant differences ($P<0.001$) among blocks and of the interaction ($p<0.001$) of blocks with provenances. Furthermore, linear regression of diameter against height (for all provenances) were found statistically significant with $P<0.01$ and $r^2 = 0.29 – 0.30$. In a relevant study on *Fraxinus excelsior,* early results of provenance trials at sites in England and Wales (Cundall et al., 2003) and also in other studies (Cicek and Yilmaz 2002, Fraxigen 2005) or combination of growth and resistance to ash dieback (Pliura et al., 2014) demonstrated significant differences among provenances in growth characteristics which supports the findings of this study. The research continues, whereas at the same time the plantation functions as *ex situ* conservation of genetic resources of *F. angustifolia* as well as for the collection of certified reproductive material (selected).

Table.1 Geographic characteristics of the natural populations of *F. angustifolia* selected for the study

Population code	Population (Area)	Latitude (°)	Longitude (°)	Altitude (m)
08FAN	Melia	40.58.300	26.07.400	155-170
09FAN	Komotini	40.59.506	25.23.300	10-15
11FAN	Doirani	41.14.701	22.46.357	50
12FAN	Ierissos	40.27.550	23.49.000	10-20
13FAN	Omolio	39.53.575	22.37.186	16-20
14FAN	Evia	38.49.144	23.25.250	120
15FAN	Louros	39.09.190	20.45.778	50
16FAN	Kalavrita	37.56.291	22.04.093	870

Table.2 Diameter growth (DBH) comparison of the eight (8) populations of *Fraxinus angustifolia*.

Population (code)	Sample no.	Mean ± s.e. (cm)*
08FAN	60	$6,09 \pm 0,15^{ae}$
09FAN	52	$6,06 \pm 0,16^{ae}$
11FAN	56	$6,14 \pm 0,19^{ae}$
12FAN	65	$6,47 \pm 0,17^{ac}$
13FAN	61	$6,74 \pm 0,20^{ab}$
14FAN	63	$6,77 \pm 0,22^{ab}$
15FAN	55	$7,30 \pm 0,16^{b}$
16FAN	55	$5,75 \pm 0,16^{de}$
Total/mean	467	6,43 ± 0,07

* Means followed by the same letter do not differ significantly ($P>0.05$, Fisher´s Least significant difference (LSD) test).

Table 3 Comparison of means of the eight (8) populations of *Fraxinus angustifolia* for the total height.

Population (code)	Sample no.	Mean ± s.e.(cm)*
08FAN	60	349.72 ± 6.82^{a}
09FAN	52	341.30 ± 5.47^{a}
11FAN	56	327.40 ± 7.35^{a}
12FAN	65	346.90 ± 6.59^{a}
13FAN	61	352.43 ± 6.96^{a}
14FAN	63	349.50 ± 9.00^{a}
15FAN	55	349.44 ± 7.04^{a}
16FAN	55	344.84 ± 6.89^{a}
Total/mean	467	345.43 ± 2.53

* Means followed by the same letter do not differ significantly ($P>0.05$) (LSD test).

5. Vegetative propagation of *Fraxinus angustifolia*

Regeneration *F. angustifolia* it is mainly done by seeds and also by stump and root sprouts (Fraxigen 2005). A new method for propagation of the species by winter stem cuttings and possibilities for breeding resistance to diseases have been tested. The aim of this type of propagation is to multiply genotypes resistant to fungal and bacterial diseases as well as to multiply selected genotypes for wood production and ornamental purposes. One year stem woody stump sprouts were taken from 10 years old mother trees (ortets) (mother trees were cut down in January 2014) of *F. angustifolia* from an experimental plantation (FRI, Thessaloniki) in January of 2015. Cuttings (18-20 cm in length) were immediately cut from the sprouts and categorized into three (3) types according to their thickness as follows: type A: 5-10 mm, type B: 11-15 mm and type C: 16-20 mm. Cuttings were treated as following: they were dipped in tap water for an hour; base wounded to a length of 3 cm; were dipped for 1min. in a fungicide solution; and quick dipped in a commercial hormone powder (3-indole butyric acid and talc). Control cuttings (K) were 10-16 mm in thickness but were only dipped in tap water. Cuttings immediately after treatment were planted into a rooting medium containing compost and fine sand (1:1 v/v) in plastic trays and placed on the bench of the glasshouse of FRI (Fig. 4). Ten (10) replications (randomly repeated) with 20 cuttings per replication/plot for each treatment were tested. Early in April bud flushing was recorded for all treatments and results were as follows (% of planted cuttings): A=66.00, B=38.00. C=33.50 and K (control)=38.00. Results showed significant differences (ANOVA test, P<0,01) among cuttings' types with type C showing the highest bud flushing percentage and type A the lowest flushing. Flushing recording continued in April (end), May and June (2015). No correlation with callus formation (at stem base) and with rooting results was found. Final assessment (August 2015) showed low rooting values. Interestingly, the type C (16-20 mm length) cuttings, resulted in the highest rooting (up to 20%) with well developed root systems. Further research is needed to improve the method by testing various parameter (e.g. soil media, type of cuttings, microenvironment, time of the year) affecting rooting of cuttings.

Figure 4 Vegetative propagation of *Fraxinus angustifolia* using winter stem cuttings (FRI glasshouse)

6. Health status of ash species in Greece

Fungi and bacteria

Fraxinus excelsior - is a rare species in Greece and rarely found dispersed in small groups or individual trees (mixed with other broadleaved trees) in the mountains of N. Greece bordering with other Balkan countries. No records of ash dieback (caused by *Hymenoscyphus fraxineus*) infection have reported up to now. *F. angustifolia* - No serious health problems for this species and no ash dieback have been reported. Other pathogens such as *Phomopsis* spp. have been isolated from natural stands and provenance trials in the field (*F. angustifolia*). Ash cankers are often found on old trees (cause agent: insects or bacteria – e.g. *Pseudomonas* spp.).

Other pathogens (e.g. *Phomopsis* spp.) and bacterial (*Pseudomonas* spp.) cankers have been recorded for the species (unpubl. data). Attacks of a serious defoliator (*Operophtera brumata* L. - Geometridae) have been observed and recorded (years 2011, 1012 and 2013) at FRI provenance trials (Thessaloniki, Greece).

Insects

Operophtera brumata L. (Geometridae). It is a serious defoliator (attacks in April/May) and has been recorded in the years 2011, 1012 and 2013 on *F. angustifolia* in a provenance trial at F.R.I. (Forest Research Institute, Thessaloniki, Greece). The winter moth, *O. brumata*, is an economic pest of apple, blueberry, oaks, maples, basswood, ash and it is native to the Palearctic Region (https://en.wikipedia.org/ wiki/2015). Phytosanity measures such as commercial insecticides (e.g. bulldock) to fight the defoliator can work successfully (unpubl. data). Serious defoliations (by newly hatched larvae - attacks in April/May) have been observed and recorded in the years 2011, 1012 and 2013 on *F. angustifolia* in a provenance trial at F.R.I. Almost all provenances have been damaged. However, early flushing provenances, e.g. No. 12, 13 and 14, showed higher damage compared to the others (Fig. 5). At individual level, there were found trees (possibly resistant?) not damaged at all or partly damaged (30 or 50% of the total foliage). Results of this study demonstrated that genotypes with hairy leaves (previously known as *F. angustifolia* var. *holotricha* (see Taxomomy/FRAXIGEN 2005) seem to be resistant to *Operophtera* attack (no records of damage). Further research is needed to look for resistant provenances and genotypes.

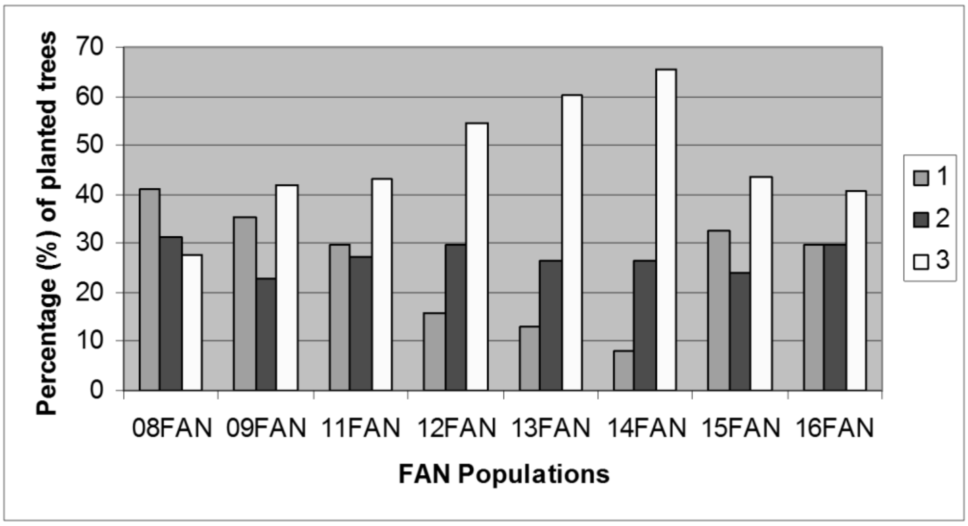


Figure 5 Provenance damage of *Fraxinus angustifolia* by the insect *Operophthera brumata* (April/May, 2011). Damage scale - 1: leaf damage <50% of the total foliage, 2: leaf damage 50-80% of the total, 3: leaf damage >80%. Y-axis shows leaf damage (%) of the total planted trees.

Leafcurl ash aphids

Prociphilus (Meliarhizophagus) sp. (Hemiptera, Aphididae, Eriosomatinae). Leafcurl ash aphids of the genus *Prociphilus* can cause significant curling and twisting of leaves which provide protection for the white waxy aphids inside (https://en.wikipedia.org/wiki/2015). Ash aphids overwinter as eggs and thus eggs hatch in the spring. Damage occurs only on new growth that emerges after the aphids arrive in the beginning of spring. Ash aphids are small light-green insects covered with off-white waxy threads. They remain on the underside of the leaves, inside the curled leaves and they feed in colonies (https://en.wikipedia.org/wiki/2015).

Even though the damage substantially disfigures leaves, it does not cause lasting damage as the leaves continue to photosynthesize the sugars the plant needs. Fully grown leaves are not susceptible to leaf curling. The damage remains confined to a very limited proportion of the tree's total foliage. However, depending on the weather conditions and the biology of the insect, sometimes the damage can be serious and thus can reduce the total photosynthesis and tree growth. Attacks are observed almost every year in the spring (April/May/June). In the year 2011, records on leaf damage of a *Fraxinus angustifolia* provenance trial (which has been described in a previous section) did not show significant differences in leafcurl damage among provenances at the age of 7 years (Fig. 6). Commercial insecticides or biological control can easily solve this problem.

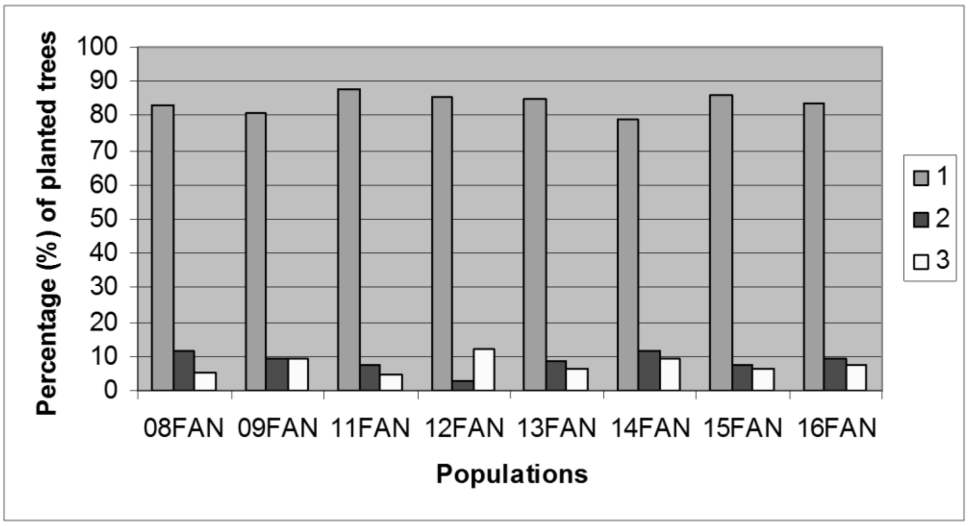


Figure 6 Leafcurl damage of *Fraxinus angustifolia* by aphids of the genus *Prociphilus*. Damage scale - 1: leafcurl damage <30% of the total crown, 2: leafcurl damage 30-60% of the total, 3: leafcurl damage >60%. Y-axis shows leafcurl damage (%) of the total planted trees.

7. Conservation measures and strategy for protecting ash genetic resources in Greece

Global climate change may cause changes of the present distribution of important species (e.g. beech, ash, elm) in Europe (e.g. movement upwards in higher altitudes) and replacing parts of the forest area by other species (e.g. oak, fir, pine). However, the following evolutionary forces should be considered when sustainable forest management and adaptive silviculture are applied on ash stands to face climate change (Pliūra and Heuertz, 2003; Boshier and Stewart, 2005; Fraxigen 2005; Spanos, 2010, Spanos et al., 2011):

Evolutionary forces

Selection – it is the only force causing adaptive evolution by acting on the adaptive traits. That explains that protection of native ash stands (particularly those of *F. angustifolia*) and *ex situ* conservation are of high importance.
Mutations - Mutations are a source of novel genetic variation, and possibly adaptive mutations are of high importance to maintain and increase adaptive potential of ash species in the long term management.
Gene flow – gene flow refers to the movement of genes through the dispersal (by air or water) of pollen and seeds.
Genetic drift – refers to the random change in allele frequency as genes transmitted from one generation to the next.

Population size

- The effective population size (*Ne*) is a fundamental parameter for conservation biology and particularly in small, isolated or fragmented populations (e.g. *F. angustifolia*).
- In large populations, stochastic genetic factors are negligible (e.g. in *F. ornus*)
- In small populations (e.g. quite often in narrow leaf ash stands in Greece), the effect of genetic factors are strong.

Threats to a population or species

Deterministic factors (they are related to anthropogenic activities)
- Deforestation (a big threat in the lowland forests of *F. angustifolia*).
- Exploitation (a big threat in the lowland forests of *F. angustifolia* and mixed stands of *F. ornus*).

- Fragmentation (a big threat in the lowland forests of *F. angustifolia*).
- Demographic and habitat alterations (a serious threat of the lowland forests of *F. angustifolia*)
- Environmental deterioration (including global climate change – in such case a population can either evolve or migrate)
- Transfer and domestication.

Stochastic factors (affect populations - particularly when small size)
Demographic stochasticity - due to variation in the survival and reproduction success of individuals (in such cases we have to secure natural regeneration of a target species in seed years - e.g. *F. excelsior*, *F. angustifolia*).
Environmental stochasticity– refers to unpredictable fluctuations in rainfall, temperature, competitors, pathogens/herbivores affecting birth and death rates.
Natural catastrophes - include fires, floods, extreme climate hazards (e.g. frost, draught, storms).
Genetic stochasticity – inbreeding depression, loss of genetic diversity, accumulation of deleterious mutations (has strong effects on very small and isolated species' populations and particularly when outside gene flow is absent) (e.g. in some very small and isolated populations of *F. angustifolia*).
To face effects of stochasticity factors, the only way is to increase population size (in this case planting with appropriate genetic material would help).

Sustainable silviculture
Climate projections predict warmer and drier conditions to come over the next few decades for the Mediterranean basin and whole Europe.

Climatic change will affect the spatial distribution of plant communities, causing a shift in vegetation patterns.
The interaction between climatic and soil factors - drought related - demonstrates that drought is a complex edaphic-climatic factor. Both components contribute to limiting the distribution of important/target forest species (e.g. *F. excelsior, F. angustifolia*)..

Actions to be taken

Forest management
- "close-to-nature forestry" - keep canopy as much as close (>0.8). It is based on the natural regeneration - provides better adaptability and sustainability (particularly in the Balkans and around Mediterranean) and low price regeneration.
- When not possible to apply natural regeneration, reforestation with suitable provenances (the most close to the local) should be applied.
- Types of fellings - most appropriate in high (seedling) forests are shelterwood and group-selection fellings (sometimes their combination).
- Clear cuts in natural forests are strongly not recommended. Avoid or reduce coppicing in *F. ornus* and *F. angustifolia* natural stands.

Conservation of forest genetic resources
- *in situ* conservation of forest genetic resources – protect old growth and remnants stands
- Virgin forests/Primeval forests,
- Special Protected areas (SPAs)
- National parks and reserves
- NATURA – 2000 areas
- Natural and semi-natural forests.
- *Ex situ* gene conservation area of (highly important for *F. angustifolia*) - seed collections in the main seed stores of each country.
- Area managed for seed production of important or threatened forest species – e.g. registered seed stands (highly important for *F. angustifolia*).

Recommendations for *in situ* and *ex situ* gene conservation of ash species in Greece and Europe:

- Further measures and actions should be taken for conservation of forest genetic resources.
- Select and use of most appropriate provenances to face climate change.

8. Conclusions

Three native Ash species are found in Greece: 1) *F. ornus* is an ecologically important species and also important for ornamental purposes. Locally it is used for making hand tools and fencing poles and may support the local economy. No serious health problems for this species and no ash dieback have been reported. 2) *F. angustifolia* is the most important ash species (in Greece) for its valuable timber and also for biomass energy use. It is also a highly important tree species for ecological restoration in the lowlands mainly in wetlands and river banks. No ash dieback records for this species in Greece have been reported. Other pathogens (e.g. *Phomopsis* spp.) and bacterial cankers (*Pseudomonas* spp.) have been recorded for this species. 3) *Fraxinus excelsior* (Common ash) is not an important tree species in Greece due to its rarity and restriction by environmental factors. No ash dieback records for this species in Greece have been reported.

Attacks of a serious defoliator (*Operophtera brumata* L. - Geometridae) have been observed and recorded (in years 2011, 2012 and 2013) at FRI *F. angustifolia* provenance trial. Almost all provenances have been damaged but early flushing provenances showed higher damage in comparison to the others.

Leafcurl ash aphids of the genus *Prociphilus* can cause significant curling and twisting of leaves of *F. angustifolia* in spring and early summer time (April/May/June). In the year 2011, records on leaf damage of the *F. angustifolia* provenance trial (7 years old) did not show significant differences in leafcurl damage among provenances.

A study of genetic variation of *F. angustifolia* natural populations was carried out in the year 2003. For the purpose of the work, eight (8) native populations of *F. angustifolia* were identified and selected all over continental Greece. Selected molecular markers of nuclear microsatellite DNA and chloroplastic DNA (cpDNA) were used to study haplotype diversity, total gene diversity, heterozygosity levels within populations and genetic diversity within and among populations. The study showed: 1) populations of narrow-leaved ash in Greece harbour most of its European cpDNA haplotypes and can be thus considered “hotspots” of its genetic diversity. 2) Relatively lower values of mean observed heterozygosity (Ho) than the mean expected heterozygosity (Hs) found in the populations and molecular markers indicate the presence (and thus also tolerance) of certain inbreeding rate by the species. 3) Genetic differentiation among populations was found low, fact which explains only a small proportion of the total genetic diversity.

Propagation of *F. angustifolia* by stem cuttings and possibilities for breeding resistance to diseases has been tested. No successful results (rooting up to 20% rooting) were obtained in this study. Further research is needed to improve this method and increase rooting percentage.

A provenance trial of the native forest tree *F. angustifolia* represented by 8 provenances has been established in Greece (FRI). Its results at the age of 6 years showed significant differences for diameter (DBH) among the tested provenances but not for their mean height.

Conservation measures and strategy for protecting ash resources in Greece are presented and practical recommedetions are cited.

References

Bakys, R. 2013. Dieback of *Fraxinus excelsior* in the Baltic Sea Region - Associated Fungi, Their Pathogenicity and Implications for Silviculture. PhD Thesis, Faculty of Natural Resources and Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences.

Boshier, D. and Stewart, J. 2005. Low local is local? Identifying the scale of adaptive variation in ash (Fraxinus excelsior) – results from the nursery. Forestry 78, 135-142.

Calama, R., Tome, M., Sanchez-Gonzalez, M., Miina, J., Spanos, K.A., Palahi, M. 2010. Modelling non-wood products in Europe: a review. *Forest Systems* 19 (SI), 69-85.

Cicek, E., and Yilmaz, M. 2002. The importance of *Fraxinus angustifolia* subsp. *oxycarpa* as a fast growing species for Turkey. In M. Ercan, A. Diner, AS Birler, C. Goulding and T. Zoralioglou (ed.) Management of Fast Growing Plantations. IUFRO Meeting/Div. 4.04.06, 11-13 September 2002, Izmit, Turkey, p. 192-200.

Cundall, E.P., Cahalan, C.M., and Connolly, T. 2003. Early results of ash (*Fraxinus excelsior*) provenance trials at sites in England and Wales. *Forestry* 76 (4): 385-400.

Dafis, S., Papastergiadou, E., Georgiou, K., Babalonas, D., Georgiadis, T., Papageorgiou, M., Lazaridou, T., Tsiaousi, V. 1997. Directive 92/43/E.C. Works biotopes in Greece: Network NATURA 2000. Gouladri Museum of Natural History – Hellenic Center of Biotopes and Wetlands, pp. 932. (in Hellenic).

Domee, B., Geslot., A., Thompson, J.D., Reille, M., Denelle, N. 1999. Androdioecy in the entomophilous tree *Fraxinus ornus* (Oleaceae). *New Phytologist* 143, 419-426.

FRAXIGEN 2005. Ash species in Europe: biological characteristics and practical guidelines for sustainable use. Oxford Forestry Institute, University of Oxford, UK, 128 pp.

Heuertz, M., Fineschi, S., Anzidei, M., Pastorelli, R., Salvani, D., Paule, L., Frascaria-Lacoste, N., Hardy, O.J., Vekemans, X. and Vendramin G.G., 2004. Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior*) in Europe. Molecular Ecology 13, 3437-3452.

https://en.wikipedia.org/wiki/Winter_moth (2015).

https://en.wikipedia.org/wiki/Prociphilus (2015).

Kirisits, T., Matlakova, M., Mottinger-Kroupa, S., Cech, T.L., Halmschlager, E. 2009. The current situation of ash dieback caused by *Chalara fraxinea* in Austria. In: Proceedings of the Conference of IUFRO Working Party, Egirdir, Turkey. *SDU Faculty of Forestry Journal*, Serial: A, Special Issue: 97-119.

Kirisits, T., Matlakova, M., Mottinger-Kroupa, S., Cech, T.L., Halmschlager, E. 2010. *Chalara fraxinea* associated with dieback of narrow-leafed ash (*Fraxinus angustifolia*). *Plant Pathology* 59: 411.

Kowalski, T. 2006. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland. *Forest Pathology* 36: 264-270.

NATURA 2000. Technical cartographic guide – NATURA 2000 Network, Thessaloniki, Greece.

Papi, R.M., Spanos, K. and Kyriakidis, D.A. 2003. Genetic diversity in *Fraxinus ornus* and *Fraxinus angustifolia* populations based on microsatellite markers. Hellenic Society of Biochemistry & Molecular Biology. Proceedings of the 55th meeting, Athens 50: 534-537.

Papi, R.M., Spanos, K.A., and Kyriakidis, D.A. 2012. Genetic variation *Fraxinus* angustifolia natural populations in Greece based on nuclear ad chloroplast microsatellite markers. *European Journal of Forest Research* 131:1151-1161.

Pliūra, A. and Heuertz, M. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for common ash (*Fraxinus excelsior*).IPGR Institute, Rome, Italy, 6 pp.

Pliūra, A., Lygis, V., Suchockas, V. and Bartkevičius, E. 2011. Performance of twenty four European *Fraxinus excelsior* populations in three Lithuanian progeny trials with a special emphasis on resistance to *Chlara fraxinea*. *Baltic Forestry* 17 (1): 17 – 34.

Spanos, K.A. (2010): (Ed.). Beech Genetic Resources for Sustainable Forestry in Europe. Proceedings of the workshop and MC Meeting of the COST Action E52. 'Evaluation of Beech Genetic Resources for Sustainable Forestry', Thessaloniki, May 5-7, 2009, NAGREF-Forest Research Institute, 133 pp.

Spanos, K.A. and Gaitanis, D. 2015. Provenance research of the indigenous forest species *Fraxinus angustifolia* Vahl. – Biometric results. 15th Conference of the Hellenic Scientific Plant Breeding Society, October 2014, Larisa, Greece, p 215-221.

Spanos, K. A., Feest, A., Petrakis, P., Daskalakou, E. 2010. Silent points on the assessment and monitoring of biodiversity. *Bioremediation, Biodiversity and Bioavailability 4 (Special Issue 1)*, 1-7. (2010) Global Science Books.

Spanos, K., Grigoriadis, N., Meliadis, I., Gaitanis, D., Grigoriadis, S., 2012. The importance and conservation values of beech forests in Greece - case study of an ancient beech forest in the NATURA area ‘Koula - Haidou’ in Xanthi, N. Greece. in: (Hans D. Knapp and Andreas Fichtner Eds.) Beech Forests - Joint Natural Heritage of Europe (2), BfN-Skripten 327, p. 141-163.

Spanos, K.A., Papi, R.M., Mylonas, D.P., Gaitanis, D. and Kyriakidis, D.A.. 2004. The European Research Programme FRAXIGEN: ash for the future, defining ash populations for conservation, regeneration and ecological adaptation - works in Greece. IUFRO Joint Conference of Division 2 – Forest Genetics and Tree Breeding in the Age of Genomics: Progress and Future, November 1-5, 2004, Charleston, South Carolina, USA, Proc.: 421-429.

Verdu, M., Spanos, K.A., Canova, I., Slobodnik, B. and Paule, L. 2007. Similar Gender Dimorphism in the Costs of Reproduction across the Geographic Range of *Fraxinus ornus*. Annals of Botany 99:183-191.

Wallander, E. 2001.Evolution of wind-pollination in *Fraxinus* (Oleaceae) – an ecophylogenetic approach, PhD Thesis, Botanical Institute, Göteborg University, Göteborg, Sweden.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 284 – 287.

Fraxinus angustifolia and forest health in Portugal - an overview

H. BRAGANÇA[1], M. C. VARELA[2]

[1] INIAV, Instituto Nacional de Investigação Agrária e Vererinária, I. P., Av. da República, Quinta do Marquês, 2780-159 Oeiras, [2] Forest researcher, senior researcher retired from INIAV.
** Corresponding author: helena.braganca@iniav.pt*

Abstract
The noble hardwood *Fraxinus angustifolia* occurs presently in Portugal mainly as riparian, ruderal and ornamental. In Portugal this species has been relatively free of disease problems despite some reports of diseased trees in urban areas. Regarding the most important species in Portuguese forests (eucalyptus, cork oak and maritime pine), during the last century changes in forest constitution and cultural practices but also external factors have increase the susceptibility to pests and pathogens. An overview of the importance of *Fraxinus* in Portugal and major potential threats for Portuguese forest is presented.

Key words: *Botryosphaeriaceae, Eucalyptus globulus, Fraxinus angustifoilia,* genetic resistance, *Quercus suber, Pinus pinaster,* Pine wood nematode, Pitch canker.

The dominant Portuguese forest types, in terms of surface, is eucalyptus (26%), followed by cork oak (23%) and maritime pine (23%) (ICNF 2013). Nevertheless other broadleaved trees are also important considering its ecologic and/or economic value, especially chestnut in the northern regions of Portugal.

The noble hardwood *Fraxinus angustifolia* Vahl. Família: Oleaceae occurs presently in Portugal mainly as riparian, ruderal and ornamental. Rural abandonment is offering recovering opportunities and profuse recolonization is seen in some zones. Climate change, land use, pests and diseases are the major potential threats for the species. The wood was valued for high quality furniture when familial woodwork industries were important in Portugal. The species was also valued as cattle fodder during summer and the bark is used on popular medicine for gout disease, rheumatism, wound healing and fever.

Fraxinus angustifolia riparian strips provide zones of positive fragmentation on sclerophyllous Mediterranean forests (Figure 1). Furthermore they also hold a variety of ecosystem services such as wildlife habitat and niche, stabilization of water streams banks against Mediterranean torrential devastating rain events, slowing zones on forest fires, diversifying of rural landscape and recreational opportunities.

In Portugal *Fraxinus* species have been relatively free of disease problems in the field; however in the last years several reports of diseased trees in urban areas were noticed (Figure 2). Different fungi inside the *Botryosphaeriaceae* family were identified on ash species in Portugal, namely *Diplodia fraxini* (Fr. : Fr.) Sacc. on *Fraxinus angustifolia* and *D. mutila* (Fr.) Mont. and *D. seriata* De Not. on *Fraxinus ornus* L. (Alves et al 2014). Fungi belonging to *Cytospora* genus are also currently isolated from diseased trees; however most of these fungi are considered opportunistic pathogens that caused disease symptoms on plants under stress conditions. The real contribution of these fungi for ash decline as well as its geographic distribution, remain almost unknown in the country. The fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya, the causal agent of Ash dieback has not been reported in Portugal till this date. During the last century changes in forest constitution and cultural practices but also external factors (*eg*. forest fires and drought stress), have increase the susceptibility of Portuguese forests to pests and pathogens (Branco et al 2014).

Eucalyptus globulus, Labill. the main forest species in Portugal is an exotic, present low diversity in the country and were free from pests and pathogens for long. However in the last decades a huge amount of different pests and diseases were found in eucalyptus stands, some with strong negative impact on the production. The insect *Phoracantha semipunctata* Fab, detected in 1980s and eucalyptus snout beetle, *Gonipterus plantensis* Marelli detected in 1995 have caused considerable damage on eucalyptus production (Branco et al 2014; Reis et al. 2012). Regarding pathogens, also an extensive variety has been found, among them *Quambalaria eucalypti* (M.J. Wingf., Crous & W.J. Swart) J.A. Simpson (Bragança et al 2015), *Neofusicoccum eucalyptorum* (Crom. ter & M.J. Wingf.) (Barradas et al 2016) and *Teratosphaeria gauchensis* (M.N. Cortinas, Crous & M.J. Wingf.) M.J Wingf. & Crous (Silva et al 2015) were reported recently for first time in Portugal.

Figure 1 Riparian population of *Fraxinus angustifolia* in SE of Portugal in a temporary water line in a sclerophyllous mixed forest of *Quercus ilex rotundifolia* and *Quercus suber.*

Figure 2 *Fraxinus angustifolia* affected by fungi of Botryosphaeriaceae family - urban area of Oeiras, Portugal.

Pinus pinaster Aiton the most important pine species in Portugal has been affected by severe bark beetles attacks (eg., *Orthotomicus erosus* (Woll.), *Tomicus piniperda* (L.) *Pissodes castaneus* (DeG.), Ips sexdentatus Boern) but also by the pine defoliator, processionary moth Thaumetopoea pytiocampa Schiff. In 1999, pine wilt disease caused by the quarantine organism *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle was first reported in Portugal and Europe emerging as a major concern for pine forest in Portugal (Mota et al 1999). Regarding pathogenic fungi, *Diplodia sapinea* (Fr.) Fuckel and *Pestalotiopsis* sp. have been often associated of pine affected areas. *Dothistroma septosporum* (Dorog.) Morelet), *Lophodermium seditiosum* Minter, Staley & Millar, *Thyriopsis halepensis* (Cooke) Theiss. & Syd, *Neofusicoccum luteum* (Pennycook & Samuels), *N. australe* (Slippers, Crous & M.J. Wingf.) and *N. parvum* (Pennycook & Samuels), were also frequently isolated (Branco et al 2014). In 2009 the quarantine fungus *Fusarium circinatum* Nirenberg & O'Donnell, the causal agent of Pitch canker disease, was first reported in the country (Bragança et al. 2009). Since the first occurrence survey and eradication procedures have been taken to prevent the spread of the disease. Until 2015 the disease was restricted to a small number of nurseries and plant and seed traders.

Evergreen oaks, namely *Quercus suber* L. has been strongly affected by decline since the second half of last century, caused by a complex interaction of biotic and abiotic factors such insects, fungi and drought stress. Insects *Lymantria dispar* (Linnaeus), *Tortrix viridana* (Linnaeus) and wood borer *Platypus cylindrus* Fabr. together with fungi in the genera *Biscogniauxia* and *Botryosphaeriaceae* family (namely *Diplodia corticola* A.J.L. Phillips, A. Alves & J.Luque) and the Oomycete *Phytophthora cinnamomi* Rands have been reported from unhealthy cork oak Portuguese ecosystem. In a recent study intended to assess the distribution of *Cryphonectria parasitica* (Murril) Barr in Portugal, a new species *Cryphonectria naterciae* M.H. Bragança, E. Diogo, & A.J.L. Phillips, was detected in association of cork oak as well as also in chestnut. However its role remains unclear for both hosts (Bragança et al 2011).

In Portugal the total area of chestnut, *Castanea sativa* (Mill.), is around 41,410 ha. Since 80s the chestnut has been affected by chestnut blight, caused by *Cryphonectria parasitica* (Murrill) Barr., but since the fungal genetic diversity is not high and the hypovirus is present in the most important chestnut-growing region in Portugal, the situation is relatively controlled (Bragança et al 2007). Ink disease, caused by *Phytophthora* spp. affects trees in the country since the 19th century and the disease is strongly widespread in the country specially in chestnut orchards and nurseries of Northeastern Portugal (Martins et al 1999, Pimentel 1947). Recently a new threat arrived, the gall wasp *Dryocosmus kuriphilus* Yasumatsu, first reported in May 2014 in the Northwestern Portugal, it is responsible for the formation of galls on new buds and is a concern for chestnut producers in Portugal (EPPO 2014).

Pest and disease resistance is a process of genetic co-evolution especially important for long living immobile organisms like forest trees. Low genetic diversity is a facilitator for the attacks of alien agents and co-evolution for further process of resistance is unlike to happen in time. Management and use of genetic resources of forest species to increase the genetic diversity is therefore an important issue to address the threat coming from the alien and invasive.

Fraxinus species have a rare breeding system of androdioecy (Dommée et al 1999) (the coexistence of two genders, cosexuals and males, in a single population). In addition vegetative propagation from stump, roots, even air layering also occurs and is frequently used by forest owners to perpetuate interesting phenotypes. Rates of inbreeding/outcrossing and clonage effects on the genetic architecture of *Fraxinus angustifolia* Portuguese populations are virtually unknown.

Understanding the mating system, inbreeding and gene flow within and among *Fraxinus* species populations and its role on genetic diversity for adaptive variation is very important for further selection of appropriate seed sources, programs for genetic improvement towards resistance including hybridization and for gene conservation. Before the threat of alien diseases it is important to provide guide lines for use of Forest Reproductive Material (FRM) not only on afforestation but also on ornamental uses to avoid importing of contaminated trees. The supply of FRM is important also to increase among and within population genetic diversity.

Under the project EUFGIS-European Information System on Forest Genetic Resources a riparian population at ALTER do CHÃO is appointed as Gene Resources Conservation Unit for *Fraxinus angustifolia* *http://portal.eufgis.org/search/simple/list/?tx_wfqbe_pi1[country_name]=Portugal*

Fraxinus sp. – Perspectives

In face of the threating agent *Hymenoscyphus pseudoalbidus* efforts shall de focused on promoting high levels of genetic variability in *Fraxinus* sp. populations for further use to enhance genetic resistance. Monitoring of the health state of *Fraxinus* sp. populations including ornamental trees for early detection is a must. Additionally, awareness on the dangerous of the disease and preventing measures shall go on specially with official institutions, private nurseries, landscape managers and general public.

Acknowledgments

We thank to COST Action FP 1103 - FRAXBACK and our colleague Jorge Capelo for information about *Fraxinus* in Oeiras urban area.

References

Alves, A., Linaldeddu, B. T., Deidda, A., Scanu, B., Phillips. A. 2014. The complex of Diplodia species associated with Fraxinus and some other woody hosts in Italy and Portugal. Fungal Diversity, 67: 143-156.

Barradas, C., Phillips, A., Correia, A., Diogo E., Bragança H., Alves A. 2016 . Diversity and potential impact of Botryosphaeriaceae species associated with Eucalyptus globulus plantations in Portugal. European Journal of Plant Pathology (in press).

Bragança, H., Diogo, L. F., Neves, L., Valente, C., Araújo, C., Bonifácio, L. and Phillips, A. 2015, *Quambalaria eucalypti* a pathogen of *Eucalyptus globulus* newly reported in Portugal and in Europe, Forest Pathology, 46: 77-75.

Bragança, H., Rigling, D., Diogo, E., Capelo, J., Phillips, A., Tenreiro, R. 2011. Cryphonectria naterciae: A new species in the Cryphonectria—Endothia complex and diagnostic molecular markers based on microsatellite-primed PCR. Fungal Biology 115(9):852–861.

Bragança, H., Simões,, S., Onofre,, N., Tenreiro, R., Rigling,, D. 2007. Cryphonectria parasitica in Portugal-diversity of vegetative compatibility types mating types and occurrence of hypovirulence. Forest Pathology 37:391–402.

Branco, M., Bragança, H., Sousa, E., Phillips, A. 2014. Pests and Diseases in Portuguese Forestry: Current and New Threats - Chapter V, 117-154 pp. In "Forest Context and Policies in Portugal-Present and Future Challenges" F. Reboredo Editor. Present and Future Challenges, Series: World Forests, Vol. 19, Springer International Publishing. 239pp.

Dommée, B.,, Geslot, A., Thompson, J.,D., Reille, M.,, Denelle, N.1999. Androdioecy in the entomophilous tree Fraxinus ornus (Oleaceae) New Phytologist, 143: 419–426.

EPPO 2014 - Reporting Service no. 06

Martins, L., Oliveira, M., Abreu, C. 1999. Soils and climatic characteristics of chestnut stands that differ on the presence of ink disease. Acta Horticulturae 494:447–449.

ICNF 2013. 6º Inventário Florestal Nacional IFN6: Áreas dos usos do solo e das espécies florestais de Portugal continental. Resultados preliminares. Instituto da Conservação da Natureza e das Florestas. Lisboa, Portugal. 34 pp.

Mota, M.M., Braasch, H., Bravo, M.A., Penas, A.C., Burgermeister, W., Metge, K., Sousa, E. (1999). First report of Bursaphelenchus xylophilus in Portugal and Europe. Nematology 1:727-734.

Silva, M., Diogo, E., Bragança, H., Machado, H., Phillips, A. J. L. 2015. *Teratosphaeria gauchensis* associated with trunk, stem and foliar lesions of *Eucalyptus globulus* in Portugal. Forest Pathology. 45: 224–234.

Reis, A.R., Ferreira, L., Tomé, M., Araujo, C., Branco, M. 2012. Efficiency of biological control of Gonipterus platensis (Coleoptera: Curculionidae) by Anaphes nitens (Hymenoptera: Mymaridae) in cold areas of the Iberian Peninsula: implications for defoliation and wood production in Eucalyptus globulus. Forest Ecology Management 270:216–222.

R. Vasaitis & R. Enderle (eds), Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management, 288 – 299.

Vegetative propagation of dieback-tolerant *Fraxinus excelsior* on commercial scale

G. C. DOUGLAS[1]*, J. M. NAMARA[1], K. O'CONNELL[1], L. DUNNE[2], J. GRANT[1]

[1] *Teagasc, Agriculture & Food Development Authority, Kinsealy, Dublin 17, Ireland,* [2] *L&K Dunne Nurseries, Kilmeague, Naas, Co. Kildare, Ireland*
** Corresponding author: Gerry.Douglas@teagasc.ie*

Abstract

Ash trees which are tolerant to Hymenoscyphus fraxineus may be selected in all age classes among heavily infected populations. They may be produced also by controlled crossings of disease tolerant trees, because the genetic component of inheritance for disease tolerance is high. For mature and juvenile plant material, the deployment of disease tolerant genotypes could be potentially achieved by vegetatively propagating selected genotypes. We describe a system to vegetatively propagate selected ash genotypes and we discuss the prospects and options for using vegetative propagation on all age classes of trees. Mature trees were rejuvenated through the process of micropropagation to establish mother plants in large trays which were cut back repeatedly (hedged) to produce at least two crops of cuttings per year.

The rooting capacity of ten genotypes was tested by a commercial nursery over a period of three years, to assess the feasibility of using hedged mother plants for efficient propagation. Commercial practise was to treat cuttings with 0.25% IBA, insert them in plug pots and maintain them covered with fine plastic within low plastic tunnels in a non heated greenhouse and without supplementary heating at the cutting base. In the first year, the mean rooting rate was 53 % for the first crop of cuttings and 35 % for the second. In the second and third years the rooting rates improved to over 80% for each crop of cuttings as experience was gained in handling the material. Rooting rate varied among the genotypes.

We assessed the growth and development of micropropagated ash trees in the field from an observation clonal trial, consisting of four mature genotypes which had been established in 2002 in five replicate plots. The micropropagated trees were generally similar in height and dbh to seed derived control trees and developed normally. These observations are discussed in the context of using vegetative propagation as a tool in breeding and for the large scale deployment of ash with tolerance to H. fraxineus.

Keywords: *Fraxinus excelsior, Hymenoscyphus fraxinea, Chalara*, common ash, vegetative propagation, cuttings, rejuvenation, hedges

Introduction

The pathogen H. fraxineus has caused a pandemic of ash dieback disease in Europe. The scale of losses in forest productivity and in ecosystem functions is very great. The production of ash plants with tolerance to this pathogen will be required on a very large scale to restore European forests in the long run. It will also require that tolerant material is generated from parent material that is well adapted to the local and regional environments. Research has shown that tolerance to this pathogen is genetically determined and this opens the opportunity for selection and for breeding ash (Kjaer et al. 2012, Pliūra et al. 2011). The imperative to avoid genetic bottlenecks is discussed by Budde et al.(2016) and the breeding methodologies which are most appropriate for ash have been outlined previously; see 'Breeding methodologies' by Alfas Pliura in Douglas et al. (2013).

Many European countries are now in the process of selecting individual ash genotypes which have displayed a high tolerance to H. fraxineus over several years, within environments with a high disease pressure. The aim thereafter is to establish seed orchards by grafting scion material from selected trees, onto unselected seedling rootstocks. Grafted material has the advantage of accelerating the onset of flowering by comparison with seed derived trees. Progeny tests can reveal the value of individual parent trees from seed orchards. Also, clonal propagation can be very useful for the genetic testing of the components used to establish new orchards, and / or for rogueing existing ones, by ranking of the genotype performance (in clonal tests), and for the subsequent selection of those that are best adapted (Lindgren 2009, 2016).The clonal reproduction of ash plants is necessary to provide planting stocks for genetic testing, as mentioned above and also as a means for bulking up scarce supplies of germplasm which may be produced from controlled crosses and from the limited seed quantities produced in the early years from clonal or seedling seed orchards.

It is important that efficient systems are developed to vegetatively propagate selected genotypes of *F. excelsior* for genetic studies, clonal testing and also for large scale deployment in forests. Research on the tolerance of clonal material to H. fraxineus revealed a great variation among genotypes in disease response; none was entirely unaffected but some remained in good health (Mc Kinney et al. 2011, Lobo et al.2014). Furthermore, the tolerance phenotype was shown to be stable in diverse environments over a period of six years (Stener 2013). Stability in disease tolerance and its strong genetic control opens the potential for the development of clonal lines of ash provided the material can be propagated efficiently. It would be prudent to deploy vegetatively propagated material in the form of polyclonal mixtures and such mixtures should consist of sets of genotypes which have a high level of genetic diversity and a proven adaptation to the target ecological zone(s) (Budde et al. 2016).

The main objective of this study was to show the potential for vegetatively propagating mature ash trees on a commercial scale and the performance of micropropagated trees in the forest. In addition we discuss the options for using vegetative propagation to compliment breeding strategies and the challenges for propagating selected material in various stages of physiological maturity.

Materials and Methods

Propagation of ash clones by cuttings on a commercial nursery

The ash cuttings were produced by micropropagated plants of mature ash trees which had been maintained as hedges by pruning. The micropropagated trees were selected originally as plus trees for a genetic improvement programme and the micropropagation method was described previously (Douglas et al. 2013). After micropropagation, the plants were transferred into a standard nursery grade peat compost with Osmocote fertiliser and trace elements in large plastic crates 60 cm long x 40cm wide x 15.5 cm deep (Figure 1A). The crates had open mesh bases and sides and consisted of numerous slits, 6mm wide and 5cm long. For cutting production, the crates were maintained above ground level on open mesh wire benches to cause air pruning of emerging roots. The micropropagated plants were grown in crates, unpruned for a year after weaning and then pruned back in the second year of growth to the lowest pair of stem buds, within approximately 1.0 - 5.0 cm of soil level, (Figure 1B). Thereafter, for four years, the plants were pruned back to the lowest sets of buds, at least three times each year to form mother plant hedges (Figure 1C). The hedged plants were overwintered outside in crates (December to March) but maintained in an unheated greenhouse during subsequent summer months for cutting production (until December). Hedged mother plants were maintained fully hydrated by an automated overhead watering line in summer. The first of the annual pruning was usually done in March before bud burst when the hedged plants were brought into the unheated glasshouse. The second pruning was in mid to late May, coinciding with the first harvest of cuttings. The third pruning was in mid to late June coinciding with the second harvest of cuttings. Thereafter the hedges were allowed to grow before their removal for overwintering outside in December.

The stock of 10 clones of ash in the form of pruned hedges were transferred a commercial producer of rooted cuttings (Dunnes nursery) in early Spring before bud burst. The mother plants were approximately four to six years old plants when used for the cutting production trials on the commercial nursery. The crates of hedged plants were placed on bricks to facilitate the continued air pruning of the roots and were maintained in an unheated, shaded greenhouse. The plants were top dressed with fresh compost containing slow release fertiliser ‘Osmocote Pro’ annually and were liquid fertilised with ’ Osmocote’ as required.

At the beginning of each season (March) the shoots of the previous year were pruned back to stimulate the outgrowth for shoots from dormant buds. Cuttings were collected from all clones on the same dates, when the leaves on new shoots were fully expanded after a growth flush and when the shoots had lignified adequately in all / most of the clones. Two harvests of cuttings were made each year, the first harvest was usually in mid May but was season dependent. Turgid cuttings were collected from fully hydrated hedged plants and were inserted on the same day into compost for rooting. The cuttings consisted of two nodes and most of the cuttings had a terminal bud. The length of the cuttings was in the range 4-8cms; they were freshly trimmed at the base and dipped (to 1 cm) into ‘Chrysotop Green’ powder containing 0.25% 1BA. The rooting compost consisted of pure peat with dolomite lime (1.2 kg /m^3) and perlite (50L / m^3). Cuttings were inserted into compost in cylindrical jumbo plug pots (50 mm diameter and 60 mm long) in a paper sleeve, open at the base.

Figure 1 A) Micropropagated ash established in the trays for hedging and cutting production; **B)** emerged shoot cuttings from hedged plants; **C)** a 4 year old hedged ash plant in winter; **D)** the low tunnels used in an unheated glasshouse for rooting of cuttings in summer. **E)** Rooted cuttings in summer; **F)** Rooted apical cutting in winter; **G)** Rooted sub apical cutting in winter; **H)** rooted cuttings after one year of growth in 'Rootrainer' pots, note root emergence from the base of tray.

The rooting environment for cuttings was on the unheated floor within low tunnels in a shaded, unheated glasshouse (Figure 1D). The tunnels consisted of translucent plastic suspended on hoops; they were 1.7m wide and 0.6m at the highest point, tapering to ground level. Additional layers of translucent plastic were applied to the tunnels as required on days of intense sunshine. Trays of cuttings (94 / tray) were placed on a woven plastic fabric layer (Mypex) within the tunnels. In 2013 and 2015 an extra layer of translucent plastic was placed over the tunnels and a layer of fine clear plastic was placed directly on top of the cuttings within the low tunnels. In addition, to improve humidity of the rooting environment further, the cuttings were positioned in the centre of the low tunnels and the edge rows of trays contained hydrated plug pots but without ash cuttings. The rooting capacity of the cuttings was evaluated in early September (Figures 1F and 1G) and cuttings were then potted into large ‘Rootrainer’ pots and were ready for field planting at the end of the following year Figure 1H.

Assessment of micropropagated ash in a pilot scale clonal field trial

We established a field trial consisting of plants from five clonal lines which had been micropropagated. The test material consisted of three clones which were selected as plus trees in mature stands (clones 47, 48, 72) one mature tree which was not selected with a valuable phenotype (F5) and the juvenile clone 8x, micropropagated from a two year old sapling. The clones were micropropagated as previously described and grown in the nursery to the sapling stage (Douglas et al. 2013). The control trees were purchased as a commercial lot of saplings. Trees were planted in March / April 2002 on small mounds in a drained field of soil type brown podzolic, pH 6.8, at a spacing of 2m X 2m in five incomplete randomised blocks. The numbers of sapling trees per block were as follows: control seed derived saplings (16); clone 8x (4); clone F5 (12); clone JK49 (12); clone M72 (4); clone JK47 (4). The trees were maintained by chemical weed control. They were pruned twice to remove side branches to a height of 2.5m. Double leaders and forks were not removed so that the true stem form could be observed.

The traits measured after 14 years of growth were: total tree height (m), diameter at breast height (dbh) in cms, the length (m) of valuable timber produced (i.e. absence of stem defects), height to the first fork (m) and stem straightness using scale 1 to 5 (1= perfectly straight, 5 = crooked). Data was analysed using the Glimmix procedure in SAS 9.4. The analysis was a one-way classification with adjustments for blocking and a weighting was applied to allow for the varying numbers of measurements contributing to each plot means. Mean comparisons were made and multiplicity in the testing was taken into account by using Tukey-Kramer adjustment of the p-value. The significance level was taken as 0.05 and residual checks were made to ensure that the assumptions of the analysis (Normality, constant variance, etc.) were met.

Results

Propagation of ash clones by cuttings on a commercial nursery

Ash cuttings were collected from ten genotypes of micropropagated plants which had been maintained in a hedged condition by pruning. First rooting was observed within two to three weeks of cutting collection on the commercial nursery (Figure 1E). Table 1 summarises the overall rooting percentages obtained from two crops of cuttings within each year, over a period of three years. The dates of cutting collection were based on the observed growth and development of shoots from the hedged plants and on the extent of lignification of the shoots. In the first year (2012), the overall rooting rates were low for the first and second harvest of cuttings (35 to 53%) compared to the second and third years (81 to 94%), Table 1.

Table 1 Overall rooting capacity of cuttings of 10 clones of *F. excelsior* collected from hedges of micropropagated plants and rooted in a commercial nursery

Cutting harvest date	Number of cuttings taken	Number of cuttings rooted	% Rooted
09-June 2012	835	447	53.5
27-July 2012	723	254	35.1
24-May 2013	694	643	92.60
14-June 2013	481	454	94.38
07-May 2015	208	185	88.9
01-June 2015	292	238	81.5

The lower rooting rates in 2012 may have been due to inexperience in handling ash at the nursery i.e., collecting too many cuttings in a sub-optimal stage of development. In addition, the cuttings may have experienced insufficient humidity provided by the single layer of translucent plastic as the cover in the rooting tunnels. For the years 2013 and 2015, fewer cuttings were selected and the humidity was improved by providing a single layer of fine plastic in direct contact with the cuttings as well as an extra layer of translucent plastic as the cover in the rooting tunnels, Figure 1D.

The rooting rates obtained by the commercial nursery varied with the clone and the harvest period. For the first harvest period of each year, the best rooting year was 2013 in which all ten clones rooted at a rate of > 80%; for 2015, seven clones gave > 80% rooting and the remaining three clones rooted in the range 60 to 70% (Figure 2). For the second harvest period of cuttings in each year, the rooting rate was over 85% for all 10 clones in 2013. In 2015 rooting was > 80% for five clones and in the range of 48 to 78% for the remaining five clones (Figure 3).

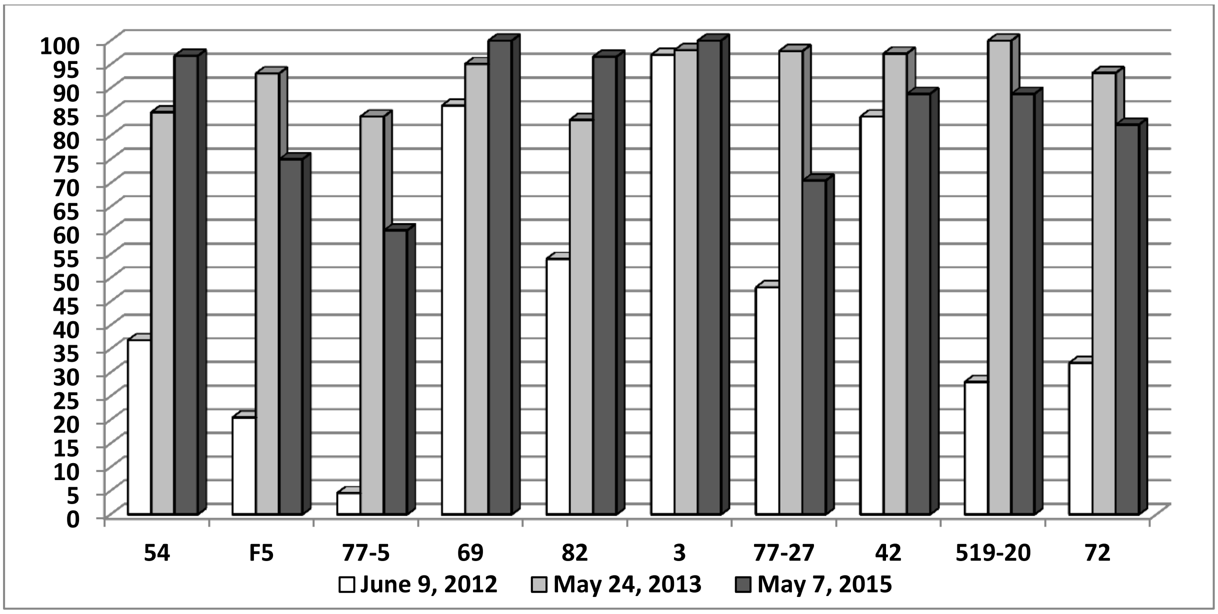


Figure 2 Rooting (%) of ash cuttings for the first annual harvest period, from hedged micropropagated plants of 10 ash clones, over three years in a commercial nursery. Clones with consistent rooting are circled.

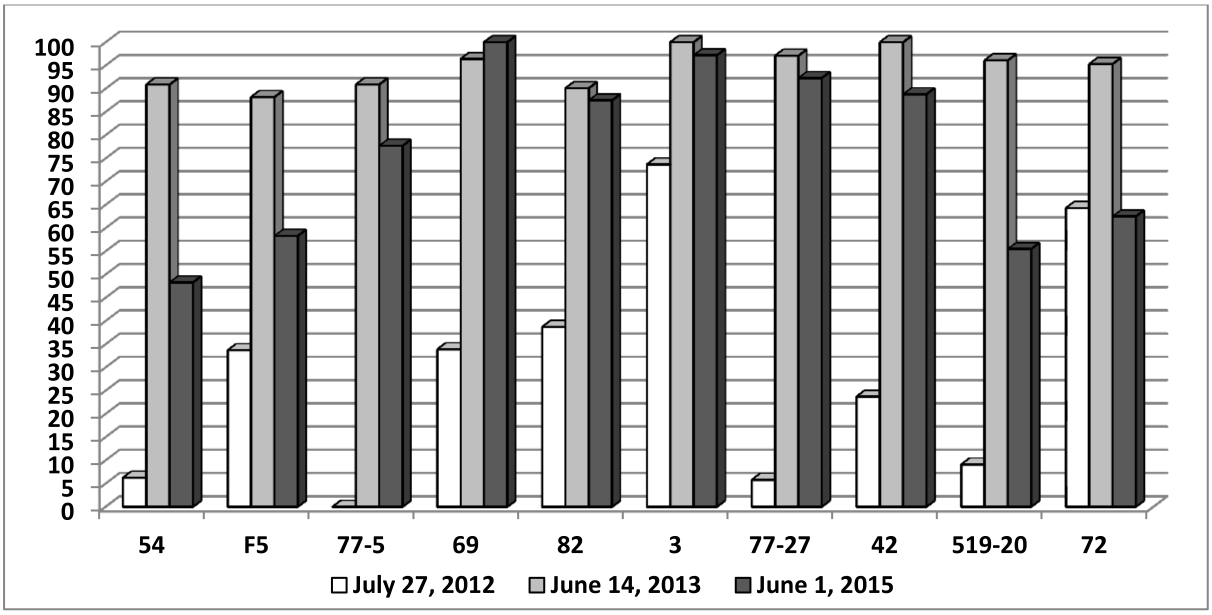


Figure 3 Rooting (%) of ash cuttings for the second annual harvest period, from hedged micropropagated plants of 10 ash clones, over three years in a commercial nursery. Clones with consistent rooting are circled.

The genotypes 69, 3, and 42 had the highest and most consistent capacity for rooting over all three years and harvest periods. In 2012, when we regarded the rooting conditions as sub optimal, these three clones gave the highest rooting rate among all clones with over 80% rooting (Figure 2). Furthermore, these same clones gave > 80 % rooting in each harvest in 2013 and 2015. All rooted plants survived transplantation into Rootrainers in early September (Figure 1F, 1G) and by September of the following year they had grown large enough for field planting (60-70 cm) Figure 1H.

Assessment of micropropagated ash in a pilot scale clonal field trial

Ash clones were planted in the field and their development was observed over a period of 14 years Figure 4. The aim of this pilot test was to record the developmental characteristics of the clones which had been produced by micropropagation compared to seed derived trees. After six years of growth, some flower production was noted and was recorded as 44% for the seedling control trees. The mean flowering frequency among the micropropagated clonal material was 41%: i.e. 82%, 96%, 8%, 0%, 21% for the clones 47, 49, 72, F5, 8x respectively.

Figure 5 The 14 year old clonal trial with micropropagated trees of *F. excelsior*

Table 2 summarises the growth and morphological characteristics after 14 years. Three of the clones were derived from plus trees and two of these performed well in growth and timber quality characteristics such as stem straightness, length of valuable timber and height to the first fork. Two other clones were derived from unselected trees (F5 and 8x) and they showed similar quality characteristics to unselected seedling derived control trees (Table 2). Overall, in terms of height growth and stem diameter the clonally produced trees grew favourably when compared to seed derived trees (Figure 4 and Table 2).

Table 2 Growth traits of micropropagated clones of *F. excelsior*: mature ash plus trees (47, 49, 72) non Plus tree clone F5, unselected juvenile clone 8x and seedling controls after 14 years of growth

Growth parameter	Micropropagated clones					Seed
	Plus trees			Unselected		Unselected
	47	49	72	F5	8x	Controls
Tree height (m)	11.1a*	11.0a	10.6a	8.1b	10.3a	10.7a
Diameter at breast height (cm)	11.4ab	11.8a	10.2ab	7.6c	9.9b	10.8ab
Valuable timber length (m)	3.0a	3.1a	0.4c	0.6c	2.1ba	1.4bc
Stem straightness (1-5; 1= excellent)	2.4d	2.1d	4.4a	4.0ab	3.2c	3.6bc
Height. to 1st fork (m)	4.5ab	4.8a	2.1c	3.2bc	3.6bac	3.5bc

*Letters in common are not significantly different, Tukey- Kramer multiple comparisons

Discussion

This study has shown that mature trees of several ash genotypes could be propagated vegetatively in a commercial nursery and that micropropagated ash trees developed normally in the field over 14 years. Chalupa (1990) was first to report the successful field establishment, overwintering and normal development of micropropagated trees of *F. excelsior*. More recent work on micropropagated clones of F. americana reported the normal development of field planted trees over a period of six years and that variation for height growth among 12 clones was twice as great as the variation within the clones (Van Sambeek and Preece 2007).

The present study shows the feasibility of producing common ash by cuttings with the potential for commercialisation. The mother plants, maintained as hedges, produced viable cuttings over several years with two harvests of cuttings per year. The economic viability of producing ash by cuttings depends on the production costs, the selling price and a high rooting percentage for a range of genotypes. The commercial nursery in this study estimated that an overall rooting rate of 80-90% would be required for a profitable operation. They also estimated that approximately 50% of the production cost would be associated with maintenance of the hedged plants. We observed some variation in the rooting capacity among the ten clones and also between the time periods and years of cutting collection. These variables may be attributed to clonal effects and to the varying physiological stage of cutting development. We obtained improved rooting rates in the second and third years of the trials by the application of an additional layer of plastic in contact with the cuttings to increase the humidity. This observation is consistent with previous work on ash cuttings which showed the importance of high humidity during rooting (Jinks 1995). At a commercial scale, a high rooting capacity in each single harvest period is highly desirable. However, different flushing times were observed among the hedged ash clones and this would probably result in each clone reaching the optimal rooting stage at different times. Research is required to optimise the culture conditions of the mother plants and to define the biochemically optimal developmental stage for enabling a high rooting capacity (de Assis et al. 2004; Schwambach et al. 2008). Further efficiencies for commercial production of ash may be possible by adapting the methods used for large scale production of eucalyptus and other tree species. With eucalyptus the intensive production of cuttings is practised using mini and micro cuttings from stock plants grown in hydroponics (de Assis et al. 2004, de Oliveira et al. 2012). For teak, the frequency of pruning the stock plants was shown to be important for both the production of cuttings and their rooting efficiency (Singh et al. 2006). Other work on eucalyptus has shown the potential of genetic markers for selecting genotypes with a high capacity for rooting (Grattapaglia et al. 1996, Marques et al. 2002).

Our starting material for cutting production was mature trees which had been previously rejuvenated through a phase of micropropagation. This system of rejuvenation resulted in cuttings which had a high rooting capacity from the hedged mother plants over several years. Using a micropropagation step to induce physiological rejuvenation has been demonstrated for shrubs such as rhododendron (Marks 1991a,b) and hydrangea (Galopin et al. 1996). Thereafter the mother plants are maintained in a juvenile state by hedging as practised with eucalyptus which are propagated on a scale of hundreds of thousands using ‘mini’ cuttings (Brondani et al. 2012, Schwambach et al. 2008). A similar process of hedging is used in operational programmes with pine (Majada 2011) and spruce (Armson et al. 1980).

Mature ash trees which display useful traits such as a high tolerance to H. fraxineus in combination with other desirable traits of apical dominance, stem form and growth rates may exist in forests throughput Europe where the disease pressure is high. This valuable material may be conserved in living collections and by cryopreservation of shoots (Schoenweiss et al. 2005) and embryos (Brearley et al. 1995). The stability of disease tolerance has been shown and in clonal material and can be exploited by vegetative propagation (Stener 2013). However, the prudent deployment of clonal selections should be in the form of large polyclonal mixtures which should have a wide genetic diversity to ensure their wide ecological adaptability (Kjaer et al. 2012, Budde et al. 2016). The use of microsatellite markers and the development of new SNP markers will facilitate the identification of sets of genotypes which would constitute a wide genetic diversity in polyclonal mixtures.

To vegetatively propagate mature ash trees by conventional cuttings, it would be necessary to first apply the rejuvenation step of micropropagation. This would generate plantlets in a juvenile state for the production of hedges that would allow the scaling up of production by using the cheaper and more efficient route of conventional cuttings. In this process the micropropagation step may prove the most challenging. Previous research on micropropagation of *F. excelsior* has concentrated on the effects of growth regulators on shoot proliferation resulting in rather low micropropagation rates in the range of 1.5 to 3.2 shoots produced per original shoot cultured / month (Douglas et al. 2013, Schoenweiss, and Meier-Dinkel 2005, Hammatt 1996, Silveira and Cottignies 1994, Pierik and Sprenkels, 1997. Similar results have been reported for other ash species: *F. ornus* (Arrillaga et al. 1992), *F. angustifolia* (Perez-Parron el al. 1994, Tonon et al. 2001a), *F. americana* (Navarrete et al. 1989) and *F. pennsylvanica* (Kim et al. 1997). The most detailed studies on *F. excelsior* have been by Schoenweiss and Meier Dinkel (2005) and Silveira and Cottignies (1994). The former reported that establishing viable shoot cultures was genotype dependent; just 20% of the genotypes from 16 year old trees were successfully established in the micropropagation phase. Furthermore, they obtained best results for initiating cultures by using emerging buds from grafted plants. Silveira and Cottignies (1994) reported that the optimal time to initiate ash culture from 4 to 7 year old trees was by using apical buds in the months of September, January or March, whereas culturing in May or June failed. Establishing aseptic shoot cultures of ash is hindered by the presence of a rich endogenous and exogenous microflora which can lead to bud necrosis (Donnarumma et al. 2011, Scholtysik et al. 2013). Other factors may be related to the micro morphology of the buds which are selected to initiate cultures (Remphrey 1989, Remphrey and Davidson 1994). It is highly desirable that more efficient methods are developed to consistently establish shoot cultures of ash which are genotype independent, especially from mature and semi mature trees for micropropagation and for rejuvenation. Using shoot meristems may facilitate the establishment of viable shoot cultures by excluding endogenous contaminants and by minimising the physiological influences of the larger tissue masses in whole buds (Ewald and Kretzschmar 1996, Ewald 1998).

Juvenile ash trees which are tolerant of H. fraxineus may be good subjects for selection, breeding and bulking up by vegetative propagation, especially if they have displayed disease tolerance over a few years, in heavily infected forests. Several studies have shown that juvenile saplings of ash are subjected to a high selection pressure and are most susceptible to infection and death by H. fraxineus. Disease pressure is highest close to ground level (Chandelier et al. 2014) and the greatest attrition rate was reported for ash trees in the age class of 5–15 years in Denmark (McKinney et al. 2011) and in Lithuania (Pliura et al. 2011). Selected juvenile trees could be transplanted from the forest to the nursery and converted to hedges as sources of cuttings by repeated pruning as described above. Ash trees generally transplant successfully because of their massive system of fibrous roots and they have a capacity for re-sprouting when cut back. Lygis et al. (2014) reported that 88.6% of stumps in the diameter class 1-10 cm regenerated shoots compared to 35.6% in the 11-20 cm class. Furthermore, previous research indicated the more juvenile nature of coppice shoots; cuttings from coppiced trees gave 47% rooting compared with 26% for shoots taken from the crowns of trees (Cahalan and Jinks 1992).

Several research groups are in the process of establishing seed orchards by selecting parent trees which display field tolerance to H. fraxineus because the heritability of tolerance is high (Pliūra et al. 2011, Kjaer et al. 2012, Lobo et al. 2014). For seed orchards, the selected genotypes are generally propagated by grafting them onto seedling derived rootstocks which are not selected. Graft viability of ash in winter and summer has given success rates of 85-97% for a diverse range of genotypes from mature trees (Douglas et al. 1996, 2001) and even for trees over 100 years old (Obdržálek and Hendrych 2014). Although this approach is conventional, some consideration should be given to the disease tolerance status of the rootstocks because emerging evidence suggests that H. fraxineus may be a causal agent in collar / root rots, either as a sole agent or in concert with other soil pathogens (Husson et al. 2012, Enderle et al. 2013, Marçais et al. 2016, Chandelier et al. 2016). It is not known if genotypes selected with tolerance to crown dieback are also tolerant of collar / root rots. Therefore, there is a case for selecting and vegetatively propagating rootstocks with known resistance to collar rots. The alternative is to select and vegetatively propagate those mature trees which appear tolerant to the combination of crown dieback as well as collar / root rots in forests where both symptoms are common. Using these trees to constitute a seed orchard would require that they would be vegetatively propagated in such a way that each tree has its own (tolerant) root system. However, rooting in the cuttings from the crowns of ash or coppice shoots is low (Cahalan and Jinks 1992) and for large scale propagation the rejuvenation step by micropropagation would be needed to obtain self rooted trees. In general it would be desirable to use genotypes with tolerance to collar rots as the rootstocks for establishing seed orchards, because they would be a potential safeguard against the development of collar rots in future years.

Outdoor seed orchards of ash will be subject to pollen inflows from sources that are situated locally (Morand et al. 2002, Thomasset et al. 2013) and far away (Bacles and Ennos 2008). These studies, using microsatellites indicate pollen flows of up to 70% and more accurate figures from multiple SNP markers will probably reveal higher figures. Consequently, ash seed orchards should be situated in sites which are surrounded by closed forest of another species, as a minimum requirement. It would be more efficient to establish indoor seed orchards to minimise pollen contamination. We have observed viable seed production in grafted trees in the greenhouse where the scion material was from adult trees. Panmixis in indoor orchards should generate ash seeds with the highest levels of tolerance to H. fraxineus. In addition, indoor seed orchards would be the most convenient for generating seed progeny from controlled crosses. In these cases the seeds produced will be valuable and in limited quantities but they could be usefully bulked up vegetatively using a variety of means as described. High rooting rates would be expected from hedges of this juvenile starting material and we estimate that such hedges should remain highly productive for at least 10 years. Micropropagation would also be an option for seed sources because seed explants are easier to establish in vitro and various seed tissues can be used to regenerate plants. Raquin et al. (2002) have demonstrated that the dormancy requirement of *F. excelsior* could be averted by culturing embryos directly from the seeds. Similarly, Van Sambeek and Preece (2007) have shown that germination in seeds of *F. americana* could be accelerated in vitro by cutting one to two mm from the end of the seeds containing the tips of the cotyledons. Shoot organogenesis has been reported in cultured embryos of *F. excelsior* and was affected by the genotype of the mother tree (Mockeliunaite and Kuusiene 2004) as well as for *F. angustifolia* (Tonon et al. 2001a). Other studies have shown that multiple plants could be regenerated from immature embryos of *F. excelsior* (Capuana et al. 2007) and their epicotyls (Mitras et al. 2009) as well as from hypocotyls of *F. profunda* (Stevens and Pijut 2012). Somatic embryogenesis may be considered as a propagation tool for seeds since the starting tissues to induce them is usually embryos in various stages of differentiation. Somatic embryogenesis has been reported for: *F. excelsior* (Capuana et al. 2007); *F. angustifolia* (Tonon et al. 2001b); *F. americana* (Preece and Bates 1995, Bates et al. 1992) and *F. mandshurica* (Kong et al. 2012; Yang et al. 2013). However, for *F. excelsior* somatic embryogenesis was confined to the seeds derived from one out of four mother trees tested and embryo induction was confined to embryos cultured at an immature stage (Capuana et al. 2007). Further development work is required to make somatic embryogenesis more efficient and applicable to a wide range of genotypes of *F. excelsior*.

Acknowledgements

We thank L & K Dunne nursery, Kilmeague, Co Kildare, Ireland for carrying out the rooting trials. We thank colleagues in the COST Action FP 1103, FRAXBACK for their very helpful collaboration.

References

Armson, K.A., Fung, M. and Bunting, W.R., 1980. Operational rooting of black spruce cuttings. Journal of Forestry, 78(6), pp.341-343.

Arrillaga, I., Lerma, V. and Segura, J., 1992. Micropropagation of juvenile and adult flowering ash. Journal of the American Society for Horticultural Science, 117(2), pp.346-350.

Bacles, C.F.E. and Ennos, R.A., 2008. Paternity analysis of pollen-mediated gene flow for Fraxinus excelsior L. in a chronically fragmented landscape. Heredity, 101(4), pp.368-380.

Bates, S., Preece, J.E., Navarrete, N.E., Van Sambeek, J.W. and Gaffney, G.R., 1992. Thidiazuron stimulates shoot organogenesis and somatic embryogenesis in white ash (Fraxinus americana L.). Plant cell, tissue and organ culture, 31(1), pp.21-29.

Beasley, R.R. and Pijut, P.M., 2013. Regeneration of Plants from Fraxinus nigra Marsh. hypocotyls. HortScience, 48(7), pp.887-890.

Brearley, J., Henshaw, G.G., Davey, C., Taylor, N.J. and Blakesley, D., 1995. Crypopreservation of Fraxinus excelsior L. zygotic embryos. Cryo-letters.

Brondani, G.E., Wendling, I., Brondani, A.E., Araujo, M.A., Silva, A.L.L.D. and Gonçalves, A.N., 2012. Dynamics of adventitious rooting in mini-cuttings of Eucalyptus benthamii x Eucalyptus dunnii. Acta Scientiarum. Agronomy, 34(2), pp.169-178.

Budde, K.B., Nielsen, L.R., Ravn, H.P. and Kjær, E.D., 2016. The Natural Evolutionary Potential of Tree Populations to Cope with Newly Introduced Pests and Pathogens—Lessons Learned From Forest Health Catastrophes in Recent Decades. Current Forestry Reports, 2(1), pp.18-29.

Cahalan, C.M. and Jiinks, R.L., 1992. Vegetative propagation of ash (Fraxinus excelsior L.) Sycamore (Acer pseudoplatanus L.) and Sweet Chestnut (Castanea sativa Mill.) in Britain. Mass production technology for genetically improved fast growing forest tree species, AFOCEL proceedings, 2, pp.371-378.

Capuana, M., Petrini, G., Di Marco, A. and Giannini, R., 2007. Plant regeneration of common ash (Fraxinus excelsior L.) by somatic embryogenesis. In Vitro Cellular & Developmental Biology-Plant, 43(2), pp.101-110.

Chalupa, V., 1990. Micropropagation of hornbeam (Carpinus betulus L.) and ash (Fraxinus excelsior L.). Biologia plantarum, 32(5), pp.332-338.

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(4) pp.289-297

Chandelier, A., Helson, M., Dvorak, M. and Gischer, F., 2014. Detection and quantification of airborne inoculum of Hymenoscyphus pseudoalbidus using real-time PCR assays. Plant pathology, 63(6), pp.1296-1305.

de Assis, T.F., Fett-Neto, A.G. and Alfenas, A.C., 2004. Current techniques and prospects for the clonal propagation of hardwoods with emphasis on Eucalyptus. Plantation forest biotechnology for the 21st century. Research Signpost, Trivandrum, India, pp.303-333.

de Oliveira, L.S., Xavier, A., Dias, P.C., Correia, A.C.G., Borges, S.R., Takahashi, E.K. and de Paiva, H.N., 2012. Rooting of mini-cuttings and micro-cuttings of Eucalyptus urophylla× E. globulus and Eucalyptus grandis× E. globulus. Scientia Forestalis, 40(96), pp.507-516.

Donnarumma, F., Capuana, M., Vettori, C., Petrini, G., Giannini, R., Indorato, C. and Mastromei, G., 2011. Isolation and characterisation of bacterial colonies from seeds and in vitro cultures of Fraxinus spp. from Italian sites. Plant Biology, 13(1), pp.169-176.

Douglas, G.C., Pliura, A., Dufour, J., Mertens, P., Jacques, D., Fernandez-Manjares, J., Buiteveld, J., Parnuta, G., Tudoroiu, M., Curnel, Y. and Thomasset, M., 2013. Common ash (Fraxinus excelsior L.). In Forest Tree Breeding in Europe (pp. 403-462). Springer Netherlands.

Douglas, G.C., Mc Namara, J. and Thompson, D., 1996. A Tube Method for Grafting Small Diameter Scions of the Hardwoods Quercus, Fraxinus, Betula, and Sorbus in Summer. In COMBINED PROCEEDINGS-INTERNATIONAL PLANT PROPAGATORS SOCIETY (Vol. 46, pp. 221-226). UNIV WASHINGTON-INT PLANT PROPAGATION SOC.

Douglas, G., Thompson, D., Harrington, F., Hennerty, M.J., Nakhshab, N. and Long, R., 2001. Vegetative propagation of selected reproductive stocks of ash and sycamore. Vegetative propagation techniques for oak, ash, sycamore and spruce, pp.16-28.

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(5-6), pp.865-876.

Ewald, D., 1998. Advances in tissue culture of adult larch. In Vitro Cellular & Developmental Biology-Plant, 34(4), pp.325-330.

Ewald, D. and Kretzschmar, U., 1996. The influence of micrografting in vitro on tissue culture behavior and vegetative propagation of old European larch trees. Plant Cell, Tissue and Organ Culture, 44(3), pp.249-252.

Galopin, G., Beaujard, F. and Gendraud, M., 1996. Intensive production of juvenile cuttings by mother microplant culture in Hydrangea macrophylla" Leuchtfeuer". Canadian journal of botany, 74(4), pp.561-567.

Grattapaglia, D., Bertolucci, F.L., Penchel, R. and Sederoff, R.R., 1996. Genetic mapping of quantitative trait loci controlling growth and wood quality traits in Eucalyptus grandis using a maternal half-sib family and RAPD markers. Genetics, 144(3), pp.1205-1214.

Harper, A.L., McKinney, L.V., Nielsen, L.R., Havlickova, L., Li, Y., Trick, M., Fraser, F., Wang, L., Fellgett, A., Sollars, E.S. and Janacek, S.H., 2016. Molecular markers for tolerance of European ash (Fraxinus excelsior) to dieback disease identified using Associative Transcriptomics. Scientific reports, 6.

Hammatt, N., 1996. Fraxinus excelsior L.(common ash). In Trees IV (pp. 172-193). Springer Berlin Heidelberg.

Husson, C., Caël, O., Grandjean, J.P., Nageleisen, L.M. and Marçais, B., 2012. Occurrence of Hymenoscyphus pseudoalbidus on infected ash logs. Plant Pathology, 61(5), pp.889-895.

Jinks, R.L., 1995. The effects of propagation environment on the rooting of leafy cuttings of ash (Fraxinus excelsior L.), sycamore (Acer pseudoplatanus L.), and sweet chestnut (Castanea sativa Mill.). New forests, 10(2), pp.183-195.

Kim, M.S., Schumann, C.M. and Klopfenstein, N.B., 1997. Effects of thidiazuron and benzyladenine on axillary shoot proliferation of three green ash (Fraxinus pennsylvanica Marsh.) clones. Plant cell, tissue and organ culture, 48(1), pp.45-52.

Kjær, E.D., McKinney, L.V., Nielsen, L.R., Hansen, L.N. and Hansen, J.K., 2012. Adaptive potential of ash (Fraxinus excelsior) populations against the novel emerging pathogen Hymenoscyphus pseudoalbidus. Evolutionary applications, 5(3), pp.219-228.

Kong, D.M., Preece, J.E. and Shen, H.L., 2012. Somatic embryogenesis in immature cotyledons of Manchurian ash (Fraxinus mandshurica Rupr.). Plant Cell, Tissue and Organ Culture (PCTOC), 108(3), pp.485-492.

Lindgren, D., 2009. Polymix breeding with selection forwards. Skogforsk.Arbetsrapport nr 687: 1-14.

Lindgren, D., 2016. The role of tree breeding in reforestation. Reforesta, 1(1), pp.221-237.

Lobo, A., Hansen, J.K., McKinney, L.V., Nielsen, L.R. and Kjær, E.D., 2014. Genetic variation in dieback resistance: growth and survival of Fraxinus excelsior under the influence of Hymenoscyphus pseudoalbidus. Scandinavian Journal of Forest Research, 29(6), pp.519-526.

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(3), pp.501-510.

Majada, J., Martínez-Alonso, C., Feito, I., Kidelman, A., Aranda, I. and Alía, R., 2011. Mini-cuttings: an effective technique for the propagation of Pinus pinaster Ait. New Forests, 41(3), pp.399-412.

Marçais, B., Husson, C., Godart, L. and Caël, O., 2016. Influence of site and stand factors on Hymenoscyphus fraxineus-induced basal lesions. Plant Pathology.

Marks, T.R., 1991a. Rhododendron cuttings. I. Improved rooting following 'rejuvenation'in vitro. Journal of Horticultural Science, 66(1), pp.103-111.

Marks, T.R., 1991b. Rhododendron cuttings. II. Factors affecting rooting following micropropagation. Journal of Horticultural Science, 66(1), pp.113-118.

Marques, C., Brondani, R., Grattapaglia, D. and Sederoff, R., 2002. Conservation and synteny of SSR loci and QTLs for vegetative propagation in four Eucalyptus species. Theoretical and Applied Genetics, 105(2-3), pp.474-478.

McKinney, L.V., Nielsen, L.R., Hansen, J.K. and Kjær, E.D., 2011. Presence of natural genetic resistance in Fraxinus excelsior (Oleraceae) to Chalara fraxinea (Ascomycota): an emerging infectious disease. Heredity, 106(5), pp.788-797.

Mitras, D., Kitin, P., Iliev, I., Dancheva, D., Scaltsoyiannes, A., Tsaktsira, M., Nellas, C.H.R.I.S.T.O.S. and Rohr, R.E.N.E., 2009. In vitro propagation of Fraxinus excelsior L. by epicotyls. J Biol Res-Thessaloniki, 11, pp.37-48.

Mockeliunaite, R. and Kuusiene, S., 2004. Organogenesis of Fraxinus excelsior L. by isolated excelsior mature embryo culture. Acta Universitatis Latviensis, 676, pp.197-676.

Morand, M.E., Brachet, S., Rossignol, P., Dufour, J. and Frascaria-Lacoste, N., 2002. A generalized heterozygote deficiency assessed with microsatellites in French common ash populations. Molecular Ecology, 11(3), pp.377-385.

Navarrete, N.E., Van Sambeek, J.W., Preece, J.E. and Gaffney, G.R., 1989. Improved micropropagation of white ash (Fraxinus americana L.). USDA Forest Service general technical report NC-North Central Forest Experiment Station (USA).

Obdržálek, J. and Hendrych, J., 2014. Propagation of the valuable historic trees of Eduard Petzold by winter grafting. Canadian Journal of Plant Breeding, 2(1), pp. 28-30.

Perez-Parron, M.A., Gonzalez-Benito, M.E. and Perez, C., 1994. Micropropagation of Frazinus angustifolia from mature and juvenile plant material. Plant cell, tissue and organ culture, 37(3), pp.297-302.

Preece, J.E. and Bates, S., 1995. Somatic embryogenesis in white ash (Fraxinus americana L.). In Somatic embryogenesis in woody plants (pp. 311-325). Springer Netherlands.

Pierik, R.L.M. and Sprenkels, P.A., 1997. Micropropagation of Fraxinus excelsior L.(Common ash). In High-Tech and Micropropagation V (pp. 330-344). Springer Berlin Heidelberg.

Pliura, A., Lygis, V., Suchockas, V. and Bartkevicius, 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(1), pp.17-34.

Raquin, C., Jung-Muller, B., Dufour, J. and Frascaria-Lacoste, N., 2002. Rapid seedling obtaining from European ash species Fraxinus excelsior (L.) and Fraxinus angustifolia (Vahl.). Annals of forest science, 59(2), pp.219-224.

Remphrey, W.R. and Davidson, C.G., 1994. Shoot preformation in clones of Fraxinus pennsylvanica in relation to site and year of bud formation. Trees, 8(3), pp.126-131.

Remphrey, W.R., 1989. Shoot ontogeny in Fraxinus pennsylvanica (green ash). I. Seasonal cycle of terminal meristem activity. Canadian Journal of Botany, 67(6), pp.1624-1632.

Schoenweiss, K. and Meier-Dinkel, A., 2005. In vitro propagation of selected mature trees and juvenile embryo-derived cultures of common ash (Fraxinus excelsior L.). Propagation of ornamental plants, 5(3), pp.137-145.

Schoenweiss, K., Meier-Dinkel, A. and Grotha, R., 2005. Comparison of cryopreservation techniques for long-term storage of ash (Fraxinus excelsior L.). CryoLetters, 26(3), pp.201-212.

Scholtysik, A., Unterseher, M., Otto, P. and Wirth, C., 2013. Spatio-temporal dynamics of endophyte diversity in the canopy of European ash (Fraxinus excelsior). Mycological progress, 12(2), pp.291-304.

Schwambach, J., Ruedell, C.M., de Almeida, M.R., Penchel, R.M., de Araújo, E.F. and Fett-Neto, A.G., 2008. Adventitious rooting of Eucalyptus globulus× maidennii mini-cuttings derived from mini-stumps grown in sand bed and intermittent flooding trays: a comparative study. New Forests, 36(3), pp.261-271.

Singh, S., Bhandari, A.S. and Ansari, S.A., 2006. Stockplant management for optimized rhizogenesis in Tectona grandis stem cuttings. New Forests, 31(1), pp.91-96.

Silveira, C.E. and Cottignies, A., 1994. Period of harvest, sprouting ability of cuttings, and in vitro plant regeneration in Fraxinus excelsior. Canadian journal of botany, 72(2), pp.261-267.

Stener, L.G., 2012. Clonal differences in susceptibility to the dieback of Fraxinus excelsior in southern Sweden. Scandinavian Journal of Forest Research, 28(3), pp.205-216.

Stevens, M.E. and Pijut, P.M., 2012. Hypocotyl derived in vitro regeneration of pumpkin ash (Fraxinus profunda). Plant Cell, Tissue and Organ Culture (PCTOC), 108(1), pp.129-135.

Thomasset, M., Fernández-Manjarrés, J.F., Douglas, G.C., Bertolino, P., Frascaria-Lacoste, N. and Hodkinson, T.R., 2013. Assignment testing reveals multiple introduced source populations including potential ash hybrids (Fraxinus excelsior× F. angustifolia) in Ireland. European Journal of Forest Research, 132(2), pp.195-209.

Tonon, G., Berardi, G., Rossi, C. and Bagnaresi, U., 2001a. Synchronized somatic embryo development in embryogenic suspensions of Fraxinus angustifolia. In Vitro Cellular & Developmental Biology-Plant, 37(4), pp.462-465.

Tonon, G., Capuana, M. and Rossi, C., 2001b. Somatic embryogenesis and embryo encapsulation in Fraxinus angustifolia Vhal. The Journal of Horticultural Science and Biotechnology, 76(6), pp.753-757.

Van Sambeek, J.W. and Preece, J.E., 2007. In vitro propagation of Fraxinus species. In Protocols for micropropagation of woody trees and fruits (pp. 179-192). Springer Netherlands.

Yang, L., Bian, L., Shen, H.L. and Li, Y.H., 2013. Somatic embryogenesis and plantlet regeneration from mature zygotic embryos of Manchurian ash (Fraxinus mandshurica Rupr.). Plant Cell, Tissue and Organ Culture (PCTOC), 115(2), pp.115-125.

Sveriges lantbruksuniversitet har verksamhet över hela Sverige. Huvudorter är Alnarp, Skara, Umeå och Uppsala.
Tel: 018-67 10 00 • Fax: 018-67 20 00 • Org nr: 202100-2817
ISBN (print version) 978-91-576-9458-4 ISBN (electronic version) 978-91-576-9459-1