Performance of susceptible and tolerant juvenile *Fraxinus excelsior* infected by *Hymenoscyphus fraxineus*, the invasive pathogen causing ash dieback

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Abstract

European ash (*Fraxinus excelsior*), a tree species with both economic and ecological importance, has experienced a steady population decline in Europe since the early 1990's due to ash dieback, caused by the pathogen *Hymenoscyphus fraxineus.* In 2001, the first observations of ash dieback were made in Sweden and by 2010, ash had become a red-listed species. In its origin in east Asia, *H. fraxineus* exists on native *Fraxinus* species, such as *Fraxinus mandshurica,* mainly as a benign associate causing little to no damage, suggesting a balanced equilibrium between host plant and pathogen. In Europe, *H. fraxineus* infects *F. excelsior* trees of all sizes, however there are several reports indicating large genotypic variation in the level of disease susceptibility among different individuals. The aim with this thesis is to evaluate heritability of ash tolerance to *H. fraxineus* by studying young progeny and clonal trials. The results aim to elucidate differences in susceptibility to ash dieback among individuals. This study has included a progeny trial, located in Alnarp, planted in 2014 with progeny from both known susceptible and tolerant mother trees. During 2014-2016, assessments were conducted to determine any variability among selected progeny in leaf phenology (bud burst), tree health status and stem form. Included in this thesis was also, a trial of selected ash genotypes planted in 2016, in Snogeholm, consisting of 65 clones, including susceptible and tolerant *F. excelsior* individuals and Asian *Fraxinus* species. In this trial, only health assessment was carried out. The results show that none of the ash families were unaffected by *H. fraxineus* but showing differences in disease severity. However, progeny from tolerant mother trees had higher survival, lower mean health class rating (HCR) and in general better stem form. This work is a first step to enable targeted genotypes to be selected for further commercial propagation, breeding and future establishment of new seed orchards.

Keywords: ash dieback, *Fraxinus excelsior, Hymenoscyphus fraxineus,* disease tolerance, heritability.

Sammanfattning

Asken, *Fraxinus excelsior,* ett trädslag som är viktigt både ur ett ekonomiskt och ekologiskt perspektiv har sedan tidigt 1990-tal tydligt minskat i antal i Europa. Detta på grund av askskottsjukan, en sjukdom orsakad av patogenen *Hymenoscyphus fraxineus*. I Sverige fann man de första skadorna orsakade av sjukdomen 2001 och asken blev rödlistad 2010. *H. fraxineus* finns på inhemska *Fraxinus* arter som t.ex. *Fraxinus mandschurica* i Asien, men då oftast utan att skada trädet. Detta indikerar att det finns en utvecklad balans mellan värdväxt och patogen. För *F. excelsior* är läget ett annat, träd i alla storlekar blir infekterade av *H. fraxineus***.** Men det finns rapporter som indikerar på en stor genetisk skillnad i mottaglighet av sjukdomen mellan olika individer. Målet med denna studie var att utvärdera askens tolerans mot *H. fraxineus* utifrån unga avkomme- och klonförsök. Resultaten syftar till att belysa olika individers tolerans mot askskottsjukan. Studien inkluderar ett försök, planterat i Alnarp 2014, med avkommor från både toleranta och mottagliga moderträd. Under åren 2014–2016 bedömdes knoppsprickning, hälsostatus, och flerstammighet för att utvärdera eventuella skillnader mellan de olika avkommorna. I studien ingick även ett försök med askkloner. Detta försök planterades 2016 i Snogeholm och bestod av toleranta, mottagliga inhemska samt asiatiska *Fraxinus* kloner. Inventeringarna utgjordes här endast av en bedömning av hälsotillståndet. Resultaten visar att ingen av askfamiljerna var helt opåverkade av *H. fraxineus* men graden av skada skiljer olika familjer. Resultaten indikerar även att avkommor från toleranta moderkloner har högre överlevnad, lägre genomsnittlig hälsoklass (HCR) och generellt mindre tendens till flerstammighet. Studien är ett första steg för att möjliggöra fortsatt urval av toleranta individer för kommande förädlingsarbete och etablering av nya fröplantager.

Nyckelord: askskottsjuka, *Fraxinus excelsior, Hymenoscyphus fraxineus,* sjukdomstolerans, ärftlighet.

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Introduction

European ash (*Fraxinus excelsior*) has experienced a steady population decline since the early 1990's, due to the lethal disease ash dieback caused by *Hymenoscyphus fraxineus*, formerly *Hymenoscyphus pseudoalbidus* (Kowalski et al. 2016). The first reports of damage caused by the disease in Europe originated from Poland in 1992 and in Lithuania in 1996 (McKinney et al. 2014, Gross et al. 2014). Currently, the pathogen is found throughout most of the natural range of *F. e*xcelsior in Europe. The first observation of damage caused by ash dieback in Sweden was in 2001 (Johansson et al. 2009) and within just a few years it had spread throughout most areas where ash is grown in the southern half of the country. The main pathway for spreading is via airborne spores (Timmerman et al. 2011). Other possible pathways may include via imports and trade, with e.g. infected seedlings, plant material (Gross et al. 2014, Timmerman et al. 2011) and potentially seeds (Cleary et al. 2013a). Globalization has allowed for an explosion in trade and unrestricted movement of various wood products and plant materials (including live plants) which are now more easily distributed around the world, though at a cost of introducing new pests and pathogens (McKinney et al. 2014), *H. fraxineus* being one of them.

H. fraxineus is native to Asia and therefore considered an invasive species to Europe which can explain its rapid spread across Europe on a naïve host population (Zhao et al. 2013, Pliura et al. 2014, Kirisits et al. 2012). Evidence for this has been demonstrated in studies that show a low genetic variability among and within the pathogen populations in Europe, while the genetic variation for *H. fraxineus* populations found in Japan and Far East Russia are indeed higher compared to European populations (Gross et al. 2014, Cleary et al. 2016). This has strengthened the theory of *H. fraxineus* having an Asian origin. Parallel to this work is the notion that the outcome of a host-pathogen interaction to some extent depends on the co-evolutionary history between those organisms. This has sparked a large interest in understanding the interactions and resulting health status of other *Fraxinus* species including those that are native to Asia. In Japan, *H. fraxineus,* occurs on the indigenous *Fraxinus* species such as *F. mandshurica* but there are no reports of any damage nor *H. fraxineus* being pathogenic to indigenous trees (Zhao et al. 2013). *F. mandshurica* has been introduced to Europe as an ornamental tree species planted in parks and arboreta. Asian *Fraxinus* species found in arboreta in Sweden generally show no disease symptoms*,* even though they are growing nearby severely damaged *F. excelsior* trees (Cleary et al. 2016). Similar reports have also surfaced in Denmark where Asian *Fraxinus* trees planted in an arboreta show little to no disease symptoms (McKinney et al. 2014). However, there have been reports of *F. mandshurica* showing symptoms of *H. fraxineus* infection from Estonia along with several other ornamental *Fraxinus* trees (*F. nigra, F. pennsylvanica,* and *F. americana)*. Of these, *F. americana* and *F. mandshurica* showed less severe symptoms than the other *Fraxinus* species planted (Drenkhan et al. 2010). In the native range of *F. mandshurica* in Far East Russia, evidence has been found for both its possible tolerance and susceptibility to *H. fraxineus. H. fraxineus* was found on asymptomatic trees, suggesting it behaves as an endophyte to *F. mandshurica*, then undergoes a saprotrophic phase prior to leaf senescence (Cleary et. al 2016). However, Drenkhan et al. (2016) suggest that *H. fraxineus* is pathogenic to the native *species, F. mandshurica* and *F. rhynchophylla* based on high levels of DNA from the fungus in necrotic areas of leaves and petioles, and low or no levels of DNA on asymptomatic leaves. Symptoms on *F. mandshurica* can be induced by inoculating stems with *H. fraxineus.* In an artificial inoculation experiment, it was demonstrated that *F. mandshurica* similarly can develop necrotic lesions in the stem. Clearly there is more research

needed to understand the ecological role and host interactions of *H. fraxineus* associated with *Fraxinus* species in its native origin. But since *F. mandshurica* does not always show disease symptoms in its native habitat and since the primary means of *H. fraxineus* spread is through wind-dispersed spores, this suggests that possible defense mechanisms in the primary affected tissues, namely the leaves, may play a role in the susceptibility of *F. mandshurica* (Gross et al. 2015).

The life cycle of Hymenoscyphus fraxineus

Most fungi have a sexual (teleomorphic) and an asexual (anamorphic) stages. For this ascomycete fungus *Hymenoscyphus fraxineus* is the teleomorph and *Chalara fraxinea* is the anamorph. The anamorphic stage of the fungus was first described in 2006 following isolation from diseased lesions (Kowalski 2006). Subsequently, the native saprophyte *Hymenoscyphus albidus* was mistakenly thought to be its teleomorph. New revelations surfaced when molecular analyses revealed two morphologically similar, but genetically distinct species of *Hymenoscyphus*, and subsequently the novel species was given the name *H. pseudoalbidus* (Queloz et al. 2011). Both species appear to occupy the same ecological niche, though it is now apparent that *H. albidus* is being competitively excluded by the new *Hymenoscyphus* species, becoming locally extinct in some areas (McKinney et al. 2012). Since 2014, *H. pseudoalbidus* has been renamed *H. fraxineus* (Kowalski et al. 2016).

For a disease to occur three factors need to interact: the plant, the pathogen and the environment (Figure 1). If the life cycle of the pathogen cannot be fulfilled because of, for example, inadequate environmental conditions that affect e.g. sporulation or germination upon host contact, no disease results. The interaction of all three factors (host plant, pathogen and environment) are necessary under ideal conditions for disease to develop (Manion 1991). For ash dieback those three interacting factors likely include: presence of more or less susceptible host plants, i.e. *Fraxinus* trees of non-Asian origin,more or less virulent isolates of *H. fraxineus* present in sufficient quantity and quality (inoculum potential) and suitable environmental conditions. For the latter, it refers to specific weather conditions such as temperature, moisture, and humidity which affect development of fruiting bodies (Landolt et al. 2016), sporulation, and germination and penetration of spores in the early stages of leaf infection (Cleary et al. 2013b).

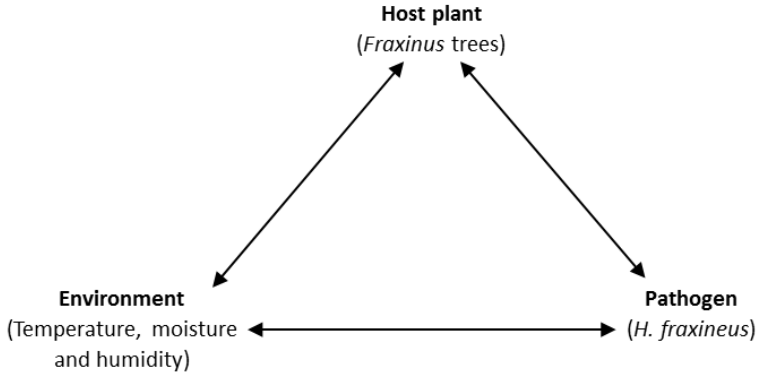

Figure 1. Disease triangle: development of diseases depends on interactions among three factors: plant, pathogen and environment.

The lifecycle of the *H. fraxineus* starts with ascospores being released and spread by wind to leaves of ash trees (Figure 2). These ascospores have been produced by apothecia grown on rachises

(≡petioles) overwintering in the leaf litter from the previous year. The spores are dispersed between June and September (McKinney et al. 2014, Gross et al. 2012). The sporulation period is largely dependent on weather conditions, and if conditions are favourable with respect to humidity and temperature, sporulation could start even earlier than June and extend into October (Timmerman et al. 2011, Gross et al. 2014). The spores land on, and start to infect, leaves and rachises of *F. excelsior* by penetrating the epidermis and leaf cuticle following appressoria formation (Cleary et al. 2013). From the entry point it is possible for the fungus to grow and spread through leaf veins to the rachises and from there it can spread further to cause necrotic lesions on the stem. Growth and spread of the fungus from individual leaflets will result in small necrotic lesions (Gross et al. 2014) which are said to be the first symptoms of ash dieback (Cleary et al. 2013b). Like all broadleaved trees, ash sheds its leaves in autumn and when this happens the fungus develops a protective black pseudosclerotial plate on rachises where it will overwinter and asexual spores are formed during that period (McKinney et al. 2014). These asexual spores have traditionally been thought to be non-infectious but rather function as fertilizing spermatia necessary for sexual reproduction. However, a recent study suggest that these spores may also be capable of germinating on leaves and cause necrosis (Fones et al. 2016). The following summer, cup-shaped fruiting bodies called apothecia are formed which release ascospores that are wind-dispersed and the life cycle of *H. fraxineus* starts again (McKinney et al. 2014, Gross et al. 2012, 2014). It is usually the year after the cycle has been completed that more pronounced disease symptoms have developed and are noticeable on the stems.

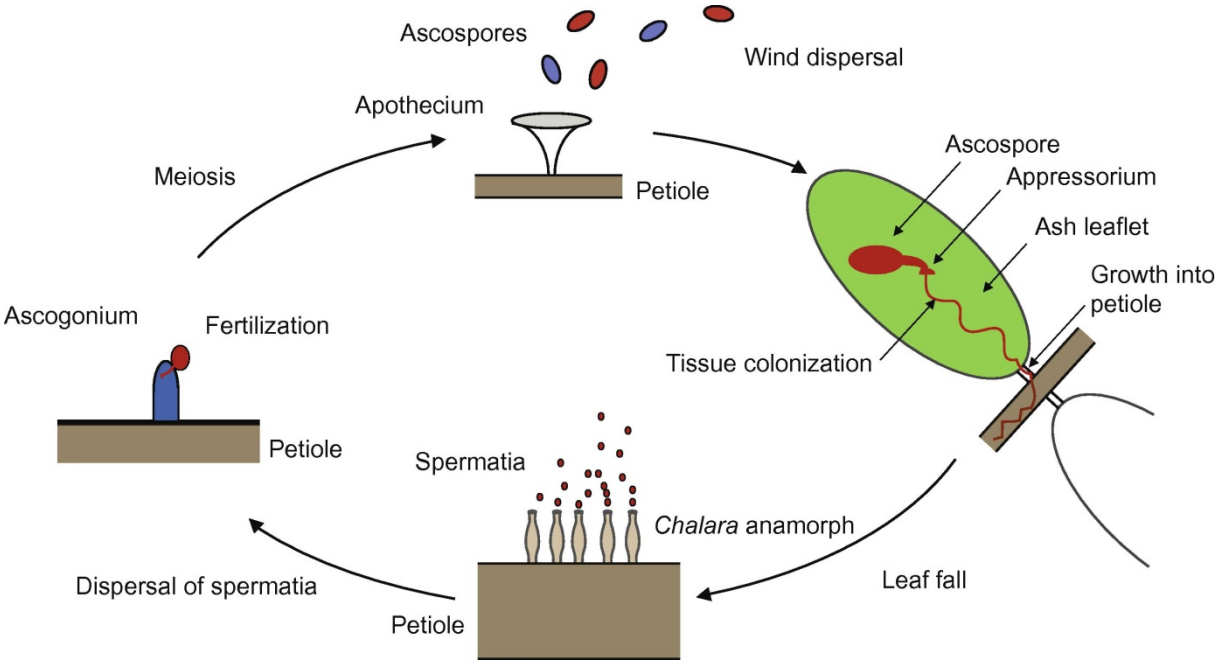

Figure 2. The hypothetical life cycle of H. fraxineus, starting with apothecia being formed and releasing wind-dispersed ascospores. Ascospores penetrate the leaf cuticle following appressoria formation and the fungus spread further through the leaf veins into the rachis (≡petiole), where it will overwinter, forming a pseudosclerotial plate on its surface, and produce asexual spores (spermatia). The following summer apothecia will be formed and release ascospores (Figure derived from Gross et al. 2012).

Disease symptoms – a myriad unrestricted to plant parts

Disease symptoms on leaves start to show soon after the infection has occurred and in late summer it is possible to detect necrotic lesions on leaf veins and rachises as first symptoms (Figure 3 c, d).

Since the infection leads to girdling of rachises the leaf can wilt and this is also considered an early visible symptom (Figure 3 e). Depending on the time of year when the shoot is girdled the symptoms will be shown differently. If the shoot is girdled during late summer, the leaflets may wilt and/or soon after desiccate, but if the girdling occurs during the winter the buds on affected shoots or stems will not burst the following spring (Gross et al. 2014). Later on, when the fungus has spread from its initial entry point, the leaves and rachises, to the stem, necrotic lesions will be visible in the bark (Figure 3 f) (McKinney et al. 2014, Pliura et al. 2014, Gross et al. 2014). These are often formed at the junction of a smaller twig or branch and a larger diameter stem. Often, where the visible bark necrosis has originated from a diseased side twig, the actual underlying necrosis could be larger (internal spread) than what is visible from surface (Marčiulynienė et al. 2017, Cleary et al. 2013, McKinney et al. 2012, Gross et al. 2014). The lesions formed on branches may girdle them if branches are small enough thus blocking the flow of nutrients and water to more distal tissues. However, lesions are often colonized by other secondary fungi which can directly antagonize and/or compete with *H. fraxineus* for substrate causing the fungus to stop growing in the outer bark. Hence, the damage caused by *H. fraxineus* on a single tree is usually a cumulative effect of chronic and annual development of lesions. When branches are girdled and start to cause typical 'dieback' symptoms in the crown, the response of the infected tree is to form epicormic shoots (Cleary et al. 2013, McKinney et al. 2014). This stress response gives the tree a bushy shape and is characteristic for trees with high susceptibility to *H. fraxineus* (Figure 3 g) (McKinney et al. 2014).

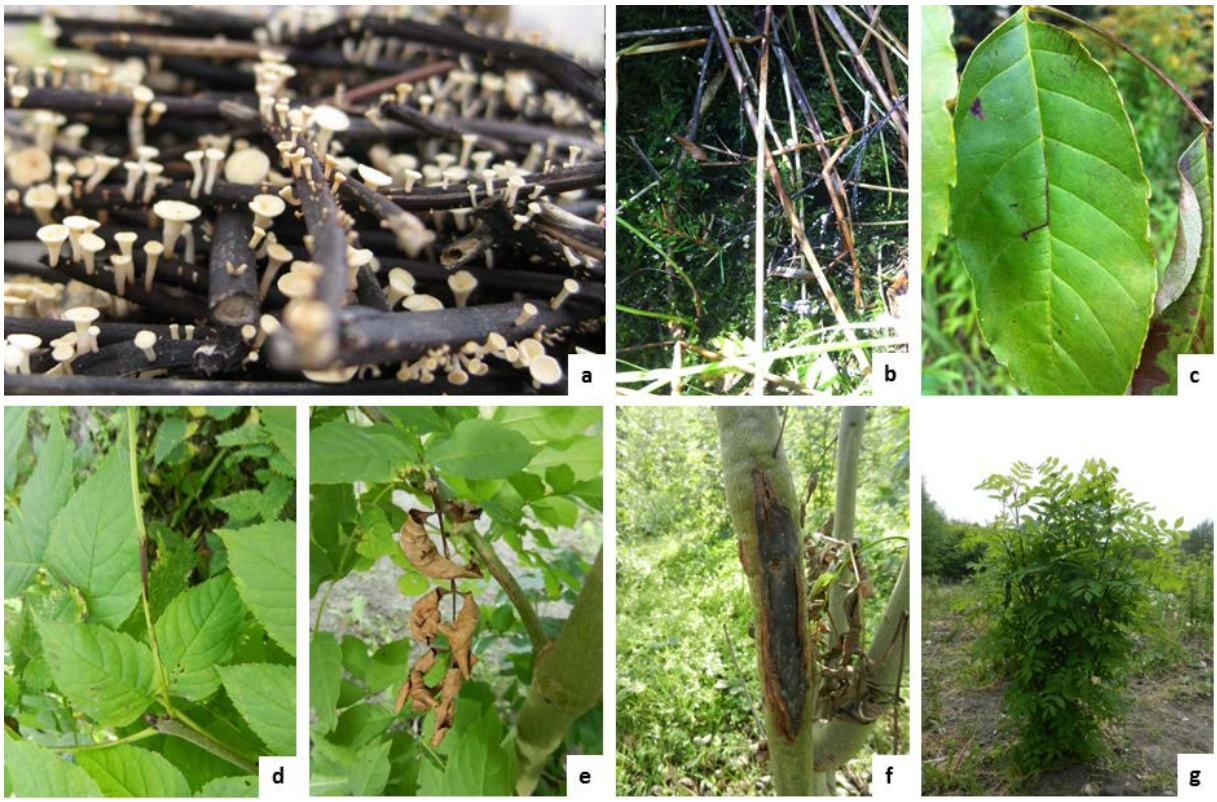

Figure 3. Signs and symptoms of ash dieback a) cup-shaped apothecia formed on black pseudosclerotial rachises, b) rachises with protective black pseudosclerotial plate on the forest floor typically in July or August, c) necrotic lesions on leaf veins, d) necrotic lesion on a single leaf rachis, e) wilted leaves resulting from current year infection, f) an older necrotic lesion in the bark of a stem that developed around a dead side twig, g) characteristic bushy shape on young ash affected by ash dieback due to killing and then resprouting of epicormic shoots. (Photos a-c: Michelle Cleary; Photos d-g: Axelina Jonsson)

***Fraxinus excelsior* – an economically and ecologically important tree species**

After the last ice age, *Fraxinus excelsior* reestablished across Europe to its most southern locations in Greece, Italy and Iran, and to its most northern location in Scandinavia (Figure 4) (FRAXIGEN 2005, Savill 2013, Skogsstyrelsen 2013, EUFORGEN 2009), through various pathways from different sources (Iberia, the Apennines, the Eastern Alps and the Balkans) which is largely reflected in their population structure. The different pathways of recolonization have resulted in populations being genetically distinct, but the studies also show that populations located close to each other have relatively low genetic diversity (Pautasso et al. 2012), meaning that for example, the populations in western and central Europe are more genetically similar to each other, while the populations in the southeastern parts of Europe are more different compared to the populations in central and western Europe. This is explained by the higher gene flow occurrence between populations in western and central Europe compared with those in southeastern Europe. The *F. excelsior* population found in Sweden is more genetically similar to the populations in the southeast of Europe (FRAXIGEN 2005), which suggests a recolonization from the south and southeast (Skogsstyrelsen 2013). It is recently found that the migration of *F. excelsior* from the south to the north was through a single pathway, explaining the genetic similarities between the two

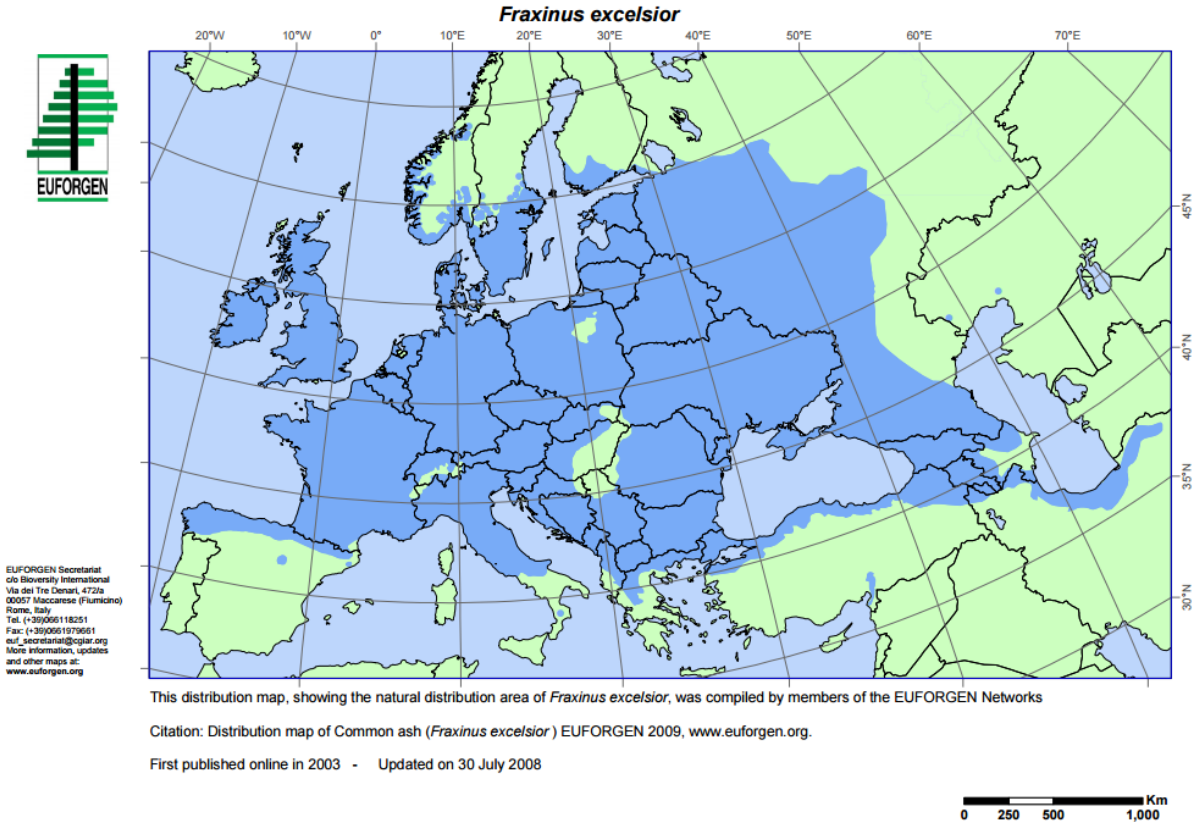

Figure 4. Distribution of Fraxinus excelsior, most southern locations in Italy, Greece and Iran and most northern locations in Norway, Sweden and Finland (EUFORGEN 2009).

populations (Tollefsrud et al. 2016).

In Sweden, *F. excelsior* is found in the south parts of the country and has its natural northern limit at *Limes norrlandicus* (Diekmann 1999, Skogsstyrelsen 2013), like many of the other broadleaf tree species in Sweden. The broadleaves in Sweden constitute approximately 18 percent of the total volume of standing stock and a small fraction of these percentages (only about 0.1%) is represented by *F. excelsior* (Riksskogstaxeringen 2016). The widespread decline and mortality of *F. excelsior* in Sweden has resulted in the species becoming red listed in 2010 with vulnerable status. Since 2015, the conservation status of *F. excelsior* has worsened to the category 'endangered', considered at risk of extinction in the wild. From being one of the most common noble broadleaf trees in Sweden, ash is now slowly disappearing from our landscapes and forests due to this invasive forest disease (Artdatabanken 2015).

Uses – one tree many options

Fraxinus excelsior has long been used for many things ranging from construction timber to herbal medicines (Thomas 2016). The wood properties of *F. excelsior* make it suitable for various uses since it is strong, durable, resilient and elastic. Ash is today mostly used for furniture, floorings, house interiors and tool handles (FRAXIGEN 2005), which all require specific properties that the species has. Historically, ash was used for ploughs, harrows, carriages and most other things that today are made with metal (FRAXIGEN 2005). Traditionally, ash was also valued for its ability to produce fodder for animals and pollarding of *F. excelsior* to get nutritious fodder for cattle was common (FRAXIGEN 2005, Skogsstyrelsen 2013). The practice of pollarding has created old trees with high biodiversity values since the cutting of branches leads to development of brown heart rot which results in a hollow stem which provides beneficial habitat for birds, insects and bats. Also, the structure of the old pollarded trees is important for biodiversity since they attract lichens, bryophytes, and fungi. A single *F. excelsior* tree can hold a large number of species and in Sweden it is possible for single pollarded *F. excelsior* to be denoted as a key habitat (Skogsstyrelsen 2013).

Ecological importance of ash

The tree species in general is important for biodiversity, not only on pollarded trees, and supports habitat for many species. One study showed that 953 species are associated with *F. excelsior* (Mitchell et al. 2014). The species dependency to *F. excelsior* is though variable; some species can use tree species other than *F. excelsior* while some exclusively use *F. excelsio*r thus making it an obligate association and essential for the species' survival*. F. excelsior* like all other tree species contributes to ecosystem services through its properties and characteristics. Ash differs from other deciduous tree species native to Europe when it comes to light conditions, decomposition rate and nutrient cycling. The light available for ground flora has a large impact on the understorey species composition. For example, a *F. excelsior* dominated forest which permits more light to the forest floor will comprise an understorey with more layers than a *Fagus sylvatica*-dominated forest. The decomposition of ash leaf litter is faster than other broadleaved trees such as *Quercus robur*, *F. sylvatica, Coryllus avelana, and Tilia* spp, because of its high nitrogen and low carbon content, but also because of the higher number of associated bacteria, fungal mycelia, protozoa and nematodes. The decomposition results in a low C:N ratio beneficial for the surrounding vegetation. In general, compared to the other tree species mentioned, ash litter contains higher concentrations of nutrients like Ca, Mg, N, S and K, which attract nutrient-demanding species (Mitchell et al. 2014). Thus, a decline in the host population because of ash dieback could result in a cascading effect on

other ecosystem processes and biodiversity (Pautasso et al. 2012, Jönsson et al. 2012, Thomas 2016). These cascading effects will be visible in all forest and landscape types where ash grows today: field margins, meadows, hedgerows, agrosystems, parks, gardens, tree avenues and different forest systems like floodplain forests where *F. excelsior* is a keystone species (Pautasso et al. 2012). Ash is commonly found growing on a wide range of sites as admixtures with other tree species including *F. sylvatica, Quercus petarea, Q. robur, Acer pseudoplatanus, Alnus incana* and *Alnus glutinosa* (Dobrowolska et al. 2011). The certain species composition that has been created in these habitats will change if the populations of *F. excelsior* continue to decline (Pautasso et al. 2012, Jönsson et al. 2012, Mitchell et al. 2014, Thomas 2016).

Disease resistance and susceptibility

A plant is considered '*resistant'* to a pathogen if it can limit or inhibit the infection caused by the pathogen. A host plant may be considered '*tolerant'* to a disease despite the limitation or inhibition of infection, by reducing the negative effects that the infectious pathogen has on the hosts' health and fitness (Roy et al. 2000). A susceptible host may be defined as "the capacity of that host to be infected by a pathogen" (Landolt et al. 2010). The pathogen *H. fraxineus* infects trees of all sizes, from seedlings to mature trees, though older trees appear to survive longer than younger or smaller trees (Kirisits et al. 2012, Witzell et al. 2014). Despite this variation among different age classes of trees when it comes to the level of damage trees ensue as a result of the disease, there is pointed evidence of some low level of disease resistance in natural populations of ash. It is clear from several research studies that there are also large genetic differences in the level of disease susceptibility among different individuals. Genetic differences in susceptibility to the disease can be seen in trees growing together in the same area under the same conditions (McKinney et al. 2014, Kjær et al. 2012). The genetic variation among trees in general depend on evolution, natural selection, gene flow, environmental conditions, postglacial migration and human activities (Pliura et al. 2007). Genetic variation among and within populations are of importance for all kinds of tree populations and for species adaptation to new conditions and overall survival (Pliura et al. 2007). For *F. excelsior,* evidence of strong genotypic and heritable effects for ash dieback (McKinney et al. 2014, Stener 2013), is important for its future and the survival of *F. excelsior* in landscapes and forests in Europe.

Previous studies

There are several studies around Europe that have investigated the genetic resistance of *F. excelsior* to ash dieback. In Lithuania, a study established in 2005 aimed to estimate the genetic variation in the population and to what extent resistance to the disease is genetically controlled in order to possibly select and breed resistant populations. This study was conducted in three progeny trials at three different locations using material from populations of *F. excelsior* originating from Lithuania and also from other countries in Europe including Germany, Denmark, Poland, France, Czech Republic, Belgium and Ireland. By the age of 8 years, only 10 percent of the original 27 000 seedlings representing 320 families were alive, and significant differences in susceptibility were found among the populations. The study revealed strong genetic difference in the progenies' susceptibility to the disease, both at population and family level, and that susceptibility is genetically predetermined and inheritable suggesting the possibility to select resistant populations (Pliura et al. 2011).

Another study was conducted in Denmark (McKinney et al. 2011), using *F. excelsior* trees selected and planted between 1934 and 1997, all of Danish origin. These trees were all selected before ash dieback was detected in Denmark (approximately 2003) and thus the criteria for a tree to be included in the trial was for good health and stem form. Grafts of the 39 plus-tree clones were planted at two different sites in 1998 which were later assessed to estimate the genetic variation in dieback damage. Assessments of disease symptoms conducted between 2007 and 2009 revealed large genetic variation between *F. excelsior* plus-tree clones with respect to symptom development (namely percent crown damage). It was found in the study that there was no tree that was totally resistant to *H. fraxineus*, but there were some clones obviously less affected showing some partial resistance to the disease. In the same study a negative correlation was found between trees and leaf phenology (time of flushing and/or leaf shed). In general, more severely damaged clones flushed and shed their leaves later than healthy clones and it was hypothesized that those clones with an early bud burst would be relatively less susceptible to the disease (McKinney et al. 2011). Other assessments of leaf phenology traits in various trials across Europe however do not support this idea (Skovsgaard et al. 2017).

Another Danish genetic study was based on progenies from 101 open-pollinated mother trees, originating from 14 different populations around Denmark and were all healthy when seeds were collected in year 2001. The seeds were used to produce seedlings which were planted in 2004 at two different sites. The selection of mother trees was done in 2001, before the first symptoms of ash dieback were reported for Denmark, but the trial was established just afterwards in 2004. The first assessment was conducted in 2008 and continued thereafter until 2010. Most trees were shown to be susceptible to the disease and the average tree health declined over the years. There were only progenies from one out of the 101 mother trees that had low levels of crown damage (meaning that less than 10 percent of the crown were damaged). This result was constant throughout all other assessments and the heritability for resistance against the disease was high, h^2= 0,37-0,52 (Kjær et al. 2011).

Another study was conducted based on a seed plantation in Austria that was established 1993 with 51 *F. excelsior* clones, all of local provenance. The first occurrence of ash dieback damage in the plantation was reported in 2008 and the assessment of the seed plantation was conducted during subsequent years. In 2010, 28 of the 51 clones (55%) had a mean dieback intensity below 10 percent. Four of the 51 clones (8%) had a high mean dieback intensity, greater than 40% crown dieback. These results indicated a large variation in dieback damage among different clones (Kirisits et al. 2011).

Ten years before ash dieback was first detected in Sweden in 2001, two *F. excelsior* seed orchards were established in the region of Skåne at Trolleholm and Snogeholm. These seed orchards were established with 106 plus-trees clones, i.e. trees were selected for vitality, good growth and high stem quality, from 27 different stands in southern Sweden. In 2006, 15 years after the establishment of the seed orchards, the first assessment was done to investigate the extent of ash dieback damage on selected trees (Stener 2013). Assessments were done twice in Trolleholm during 2010 and 2011, and four times in Snogeholm between 2006 and 2011, using specific damage classification for branch and crown dieback. Over a five-year period, the cumulative mortality was 33% in Snogeholm (the bigger of the two trials) equating to a loss of about 7% per year, and survival was poorly genetically controlled, H^2 = 0.09. In Trolleholm, the mortality between years was also 7%

and no significant difference in mortality was found among clones, $H^2 = 0.00$. However, the study clearly demonstrated that there is a large genetic variation in traits that are associated to ash dieback. Similarly, Stener (2013) found that no clones were unaffected by ash dieback but that there was a strong genetic variation among clones and their susceptibility to the disease, such that some few (<5%) stay relatively vital.

The earlier mentioned study from Lithuania (Pliura et al. 2011) has been the basis for further studies on the topic. The more recent study by Pliura et al. (2014) specifically aimed at evaluating any temporal changes in the genetic resistance to *H. fraxineus* and also the genetic variation and heritability of resistance in progeny. For this, 50 *F. excelsior* progenies from the previous study established as clones (Pliura et al. 2011) were chosen to be assessed in a new trial. The 50 clones were selected for their high resistance against *H. fraxineus* and were divided among Lithuanian populations and populations from other European countries including Czech Republic, France, Germany and Ireland. All clones were planted in 2012 at seven different locations. Assessments were carried out in May and August during the years 2012 and 2013. Results showed a general increase in disease incidence, from 10.7% in May 2012 to 72.2% in May 2013, and by the end of 2013 all clones showed some symptoms of ash dieback. However, disease severity varied significantly among different clones as well as populations further substantiating that damage caused by the disease is under strong genetic control (Pliura et al. 2014).

To summarize, in all the different trials assessed in different countries on trees of different ages it seems that most *F. excelsior* trees are susceptible to ash dieback (Kjær et al. 2012) and that no tree goes unaffected by the pathogen but that trees of different genetic origin show variable degrees of susceptibility to *H. fraxineus* (McKinney et al. 2011, Stener 2012, Pliura. et al 2014). The mortality caused by *H. fraxineus* in affected areas is high and the survival rate is low. For example, in Lithuania one study demonstrated 10 % survival only eight years after planting. A Danish study showed that only 5% of the planted trees remained healthy nine years after planting (McKinney et al. 2014). In all studies though there is strong evidence of variable expression of symptom development among individuals, such as bark necrosis (McKinney et al. 2011, 2014; Kjær et al. 2012) and extent of dieback intensity (Pliura et al. 2011, 2014, McKinney et al. 2011, Kirisits et al. 2011, Stener 2013). The role of leaf phenology in disease resistance though is not consistent among trials, and therefore less clear. In most of these studies it was found that the susceptibility of progeny/clones/populations/ individuals depends on host genetics (Pliura et al. 2011, McKinney et al 2011, Kjær et al. 2012, Stener 2013, Pliura et al. 2014). This information is useful for future efforts aimed at selection and breeding of ash. In particular, it is important to know if the detected resistance is inherited from the parent trees to the progeny. Narrow sense heritability for resistance in one of the Lithuanian progeny studies was shown to be high; between 0.40 and 0.49 (Pliura et al. 2011), and in the Danish progeny study these values ranged between 0.37 and 0.52 (Kjær et al. 2011). Collectively, these results suggest a significant level of additive genetic variation in resistance such that the progeny of parents with less susceptibility are able to inherit this trait. In turn, this opens a possibility for breeding more resistant trees in the future through artificial selection (McKinney et al. 2014).

Aim

The aim with this thesis is to evaluate the tolerance of selected *F. excelsior* genotypes to damage caused by *H. fraxineus*. By looking at the i) genetic variation and heritability of selected progeny of *F. excelsior* established as clones in a field trial after 3 years, and ii) the performance of selected *F. excelsior* genotypes vegetatively propagated from wild-type selections across Sweden. The results presented herein will give an early overview of genotype performance in order to detect individuals which are more genetically tolerant to *H. fraxineus*, which can be used for further breeding and future establishment of seed orchards for commercial propagation.

Material and methods

The assessments performed in this thesis were conducted in two separate field trials both located in southern Sweden: 1) a progeny trial located in Alnarp (55°39 N 13°04 E, 4 m) and 2) a clonal trial in Snogeholm (55° 32´ N, 13° 42´ E, 50 m) comprised of ash of different genetic background.

1) Ash progeny trial:

Experimental Design and Plant Material

The progeny trial at Alnarp was established in spring 2013 in the landscape laboratory at SLU, replacing a former plantation of *F. excelsior*. The previous stand was established initially in 1994 using seedlings of Swedish origin obtained from Rögle plantskola, Ängelholm, in southern Sweden (R. Overgaard, personal communication). Initial assessment of the plantation in 2011, i.e. 16-years after establishment, indicated that most trees had been killed by *H. fraxineus*, with the exception of some few having minimal or less severe crown dieback (M. Cleary, personal communication) (Figure 5).

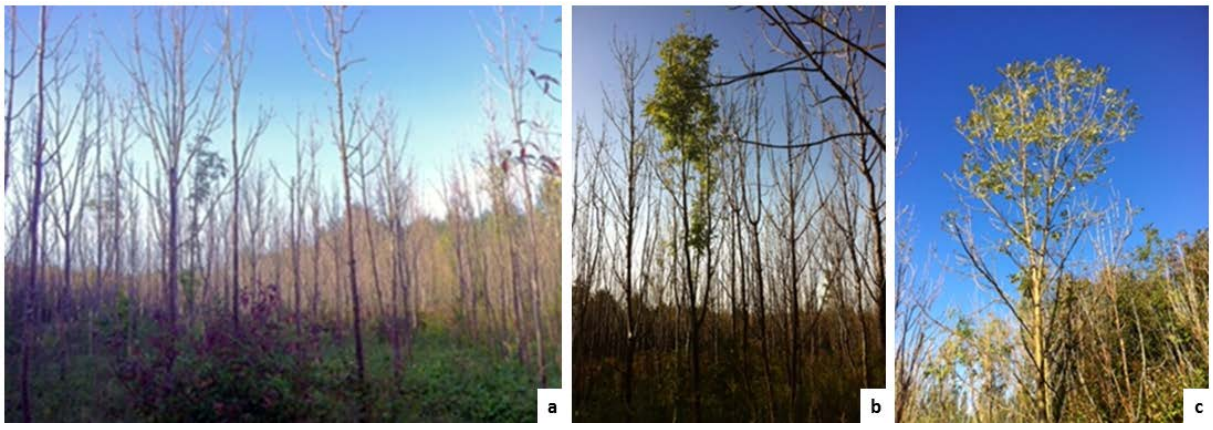

Figure 5. The previous ash stand in the landscape laboratory at Alnarp a) most of the trees were killed by H. fraxineus, b) only some few individuals displayed relatively healthy crowns, c) a tree still being alive showing crown dieback. Photos: Michelle Cleary.

In late autumn 2013, most trees were harvested, apart from some trees exhibiting remarkable tolerance to the disease. These were to be included in a new selection of promising clones being tolerant to ash dieback damage. In addition, a perimeter of dead trees close to the walking path at the border of the area, were left for demonstration purposes. After harvesting the trees, all stumps were removed and deep scarification of the soil was performed by turning top soil upside down to reduce competing vegetation (mainly grasses). A 3-metre buffer zone was placed around the

perimeter of the planted trial area. A fence was set up around the area, to prevent browsing by rabbits or deer.

The trial is comprised of seedlings originating from open-pollinated families from 13 mother clones of varying susceptibility to *H. fraxineus* according to an evaluation by Stener (2013). The thirteen families to be tested originated from seed collected from two ash seed orchards in Trolleholm and Snogeholm in southern Sweden (Table 1). There are six families where the mother clones were more notably tolerant and seven with high susceptibility, these were included as a comparable control. Seedlings were initially grown from seed via somatic embryogenesis in tissue culture according to Cleary et al. (2014) and then transferred to soil and maintained in a greenhouse. The seedlings comprised two different plant types depending on the location and the length of cultivation: plants were either propagated in Ekebo (2-year-old plants) or Uppsala (1-year-old plants). All plants were labelled by using a unique family code and by adding 100 for the Ekebo plants and 200 for the Uppsala plants, it was possible to consider the different plant types in the evaluation. Thus, the family identities 121 and 221 for instance, refers to the same family (no 21) but of two different plant types. The planting took place in spring 2014 and included a total of 300 seedlings and at least one meter spacing between trees. A random block design was used for the layout of the trial, with a total of ten plots (blocks), 30 plants per plot, and one or two plant types per family within each plot. Plants of a known susceptible genotype were fill planted around the marked plots and the perimeter of the trial. Mechanical and manual weeding was conducted several times throughout the first two years, and when needed during subsequent years.

Table 1. List of tested F. excelsior seedlings at Alnarp trial. "Seed orchard" refers to the location from where the seeds were collected.

Family number	Seed orchard	Susceptibility Rating[1]	No. of seedlings by plant type (Ekebo/Uppsala)
01	Snogeholm	Susceptible	14/10
04	Snogeholm	Tolerant	15/10
06	Trolleholm	Susceptible	21/12
12	Trolleholm	Susceptible	0/5
14	Snogeholm	Tolerant	15/14
15	Trolleholm	Susceptible	15/11
18	Snogeholm	Tolerant	18/14
21	Snogeholm	Tolerant	18/20
22	Trolleholm	Susceptible	17/4
24	Snogeholm	Susceptible	7/0
25	Trolleholm	Susceptible	18/10
28	Snogeholm	Tolerant	15/0
31	Snogeholm	Tolerant	17/0

[1]*based on initial work of Stener (2013)*

Assessments:

All assessments were carried out for each individual plant in each of the main plots and included bud flushing (the degree of flushing on a given date in early spring), a health scoring which covered the overall health status of the tree and an assessment of stem form. Bud flushing was conducted on May 21st 2016. In the previous year bud burst was assessed on May 4th. For the present study,

tree health assessments were performed in early June, late July, and late September. Height measurement of all living plants was also done in late September. The data collected during the current year 2016 was combined with data collected from two previous years, and analysed accordingly. Note: assessments from the previous years were performed by another person.

Bud flushing:

To assess bud flushing, the following scale was used as a guide to determine the degree of flushing that had occurred (Table 2). Trees were given a score based on the overall impression of bud burst for the whole tree. The scale ranged between 0 and 5 whereby the lowest score, 0, represented a bud that was still dormant and the highest score, 5, represented leaves that had fully opened from their buds and were notably expanded.

Table 2. Description of ratings used to determine stage of budburst

Rating	Description
0	The bud is dormant and black
1	The bud is green and have begun to swell
2	The leaves have begun to emerge from the bud
3	The leaves have begun to open and petioles are visible
4	The leaflets are open and petioles have begun to extend
5	The leaves are fully open and expanded

Tree health status:

The determination of the tree health was done by scoring trees into different health classes which describe the severity of damage caused by *H. fraxineus (Table 3)*. In order to place a tree into one of these categories both the number of necrotic lesions on the tree and the total length of the necrosis on the stem were measured.

Table 3. Health class ratings used to categorize the degree of damage on Fraxinus species caused by H. fraxineus

Rating	Description
1	**Symptomless,** No visible symptoms of the disease
2	**Slightly damaged,** Brown necrosis on leaves or leaf vein, wilted or dry leaves and/or a single small necrotic lesion on stem or shoot
3	**Moderately damaged,** Two or three necrotic lesions on the stem
4	**Severely damaged,** Four or more necrotic lesions on the stem or 50 percent of the tree is damaged
5	**Dead,** The main stem and all emerged sprouts are dead

Stem form:

A scoring of the stem form was performed for all trees and assigned to one of three categories (Table 4). A tree with a stem form rating of one meant that the tree has a single stem. A tree with a rating of two meant that one or two new stems has sprouted from the lower part of the stem following initial death of a main leader stem. A tree with a rating of three included those who had

three or more stems that had sprouted from the lower part of the stem; thus giving the tree a noticeable 'bushy' appearance.

Table 4. Rating for stem form divided into three categories depending on number of stems

Rating	Description
1	**1,** One single stem
2	**≤2** One or two new stems have sprouted from the lower part of the original stem
3	**≥3,** Three or more stems have sprouted from the lower part of the original stem

2) Snogeholm clonal trial

Experimental Design and Source Material

Extensive surveys for the selection of vital *F. excelsior* trees were conducted between the years 2014 and 2015 in forests and the natural landscape including key habitat areas for ash (nyckelbiotop) and known seed stands throughout the natural distribution range of ash in the southern half of Sweden. In those surveys, more than 500 vital ash trees were identified and marked for selection and further monitoring. For a tree to be considered vital and marked for selection, at least 80 percent of the crown needed to be intact. Stem quality and growth characteristics were secondary to tree vitality. From this survey 56 vital trees (clones) were selected for clonal propagation by grafting. In addition, four known susceptible mature *F. excelsior* trees and five Asian *Fraxinus* trees were selected. The Asian *Fraxinus* were selected from the botanical garden of Gothenburg and in Alnarp, SLU (Table 5) and belonged to the species *mandshurica*, *japonica* var. *stenocarpa*, *platypoda*, and *spaethiana*. These were included to give a unique comparison of symptom development on what is presumably a known host to *H. fraxineus* in its native origin of Asia (Zhao et al. 2013, Cleary et al. 2016). Scions from each selected tree were grafted onto 2-year old *F. excelsior* rootstock plants originating from a resistant genotype (mother clone) in Denmark. Up to 13 grafts (replicates) per clone were produced. The trial in Snogeholm was established using a randomized block design. A total of 65 clones (Table 5) were planted in May 2016. The clones were distributed on 12 plots (blocks), with at least one meter spacing between the clones and at least one graft per clone was randomly established in each plot.

Table 5. List of tested clones at the Snogeholm trial. The clones from Trolleholm seed orchard have been found to be tolerant in an evaluation by Stener (2013)

Clone number	Origin	Fraxinus species	Susceptibility rating[1]	No. of ramets
8	Trolleholm	*F. excelsior*	Tolerant	13
44	Trolleholm	*F. excelsior*	Tolerant	13
57	Trolleholm	*F. excelsior*	Tolerant	13
62	Trolleholm	*F. excelsior*	Tolerant	13
65	Trolleholm	*F. excelsior*	Tolerant	13
66	Trolleholm	*F. excelsior*	Tolerant	12
89	Trolleholm	*F. excelsior*	Tolerant	9
93	Trolleholm	*F. excelsior*	Tolerant	13

3001	Munkedal	*F. excelsior*	Susceptible	12
3002	Munkedal	*F. excelsior*	Tolerant	11
3003	Munkedal	*F. excelsior*	Tolerant	13
3004	Munkedal	*F. excelsior*	Tolerant	13
3005	Munkedal	*F. excelsior*	Tolerant	8
3006	Öland - Kalkstad	*F. excelsior*	Susceptible	13
3007	Öland - Ismantorp	*F. excelsior*	Tolerant	13
3008	Öland - Ismantorp	*F. excelsior*	Tolerant	12
3009	Öland - Ismantorp	*F. excelsior*	Tolerant	13
3010	Öland - Ismantorp	*F. excelsior*	Tolerant	12
3011	Öland - Ismantorp	*F. excelsior*	Tolerant	11
3012	Öland - Kalkstad	*F. excelsior*	Tolerant	13
3013	Öland - Kalkstad	*F. excelsior*	Tolerant	12
3014	Öland - Kalkstad	*F. excelsior*	Tolerant	13
3015	Öland - Kalkstad	*F. excelsior*	Tolerant	13
3016	Omberg	*F. excelsior*	Tolerant	6
3017	Jönköping	*F. excelsior*	Tolerant	12
3018	Nässjö	*F. excelsior*	Tolerant	13
3019	Nässjö	*F. excelsior*	Tolerant	7
3020	Nässjö	*F. excelsior*	Tolerant	13
3021	Omberg	*F. excelsior*	Tolerant	11
3022	Omberg	*F. excelsior*	Tolerant	13
3023	Omberg	*F. excelsior*	Tolerant	13
3024	Omberg	*F. excelsior*	Tolerant	13
3025	Öland - Löttorp	*F. excelsior*	Susceptible	12
3026	Öland - Löttorp	*F. excelsior*	Tolerant	13
3027	Öland - Löttorp	*F. excelsior*	Tolerant	13
3028	Öland - Löttorp	*F. excelsior*	Tolerant	13
3029	Öland - Löttorp	*F. excelsior*	Tolerant	12
3031	Alnarp	*F. excelsior*	Tolerant	13
3032	Alnarp	*F. excelsior*	Tolerant	9
3033	Alnarp	*F. excelsior*	Tolerant	13
3034	Alnarp	*F. excelsior*	Tolerant	13
3035	Alnarp	*F. excelsior*	Tolerant	12
3036	Sturup	*F. excelsior*	Tolerant	13
3037	Sturup	*F. excelsior*	Tolerant	13
3038	Sturup	*F. excelsior*	Tolerant	13
3039	Sturup	*F. excelsior*	Tolerant	13
3040	Sturup	*F. excelsior*	Tolerant	13
3041	Sturup	*F. excelsior*	Tolerant	13
3042	Sturup	*F. excelsior*	Tolerant	13
3043	Sturup	*F. excelsior*	Tolerant	13
3044	Sturup	*F. excelsior*	Tolerant	13
3045	Sturup	*F. excelsior*	Tolerant	13
3046	Karlsborg	*F. excelsior*	Tolerant	13
3047	Karlsborg	*F. excelsior*	Tolerant	12
3048	Karlsborg	*F. excelsior*	Tolerant	13
3049	Lysekil	*F. excelsior*	Susceptible	13

3050	Lysekil	*F. excelsior*	Tolerant	13
3051	Lysekil	*F. excelsior*	Tolerant	12
3052	Gothenburg - botanical garden	*F. japonica var.stenocarpa*	Tolerant	8
3053	Gothenburg - botanical garden	*F. mandshurica*	Tolerant	11
3054	Gothenburg - botanical garden	*F. mandshurica*	Tolerant	11
3055	Gothenburg - botanical garden	*F. platypoda*	Tolerant	5
3056	Alnarp	*F. spaethiana*	Tolerant	11
3057	Lysekil	*F. excelsior*	Tolerant	9
3058	Lysekil	*F. excelsior*	Tolerant	9

[1] based on field surveys conducted by M. Cleary and L-G. Stener during the years 2014 and 2015.

Assessments:

Since the trial in Snogeholm was established during the current year 2016, only health assessment was done and was conducted in late July and at the end of September. Disease incidence ratio and survival was calculated based on the collected data. Height measurements of the planted trees was conducted in late September.

Calculations and statistical analysis

For both locations, disease incidence ratio (the number of symptomatic seedlings divided by the total number of seedlings in each family) and survival (number of living seedlings divided by the total number of seedlings in each family) was calculated.

The statistical model for the trial in Alnarp is given as: Y = block + family + plant type + family*plant type + error. Therefore, to be able to statically test the hypothesis of this project an analysis of variance (ANOVA) was carried out for each of the different assessments: bud burst, health class rating and stem form. For the ANOVA for the mentioned design, a linear mixed-effects model was first conducted where fixed variables were block and plant type and random variables were family (among family effects) and residuals (within family affects). The significance level was set to p=0.05. To test if significant differences occurred between tolerant and susceptible families a two-way ANOVA was conducted for the assessment health class rating. The significance level was set to p=0.05. All statistical analysis was done in R, version 3.3.2. (see also Appendix I). Calculations of h^2, narrow sense heritability, were done by using the variance calculated from the standard deviation given in R and the formula used were: h^2=Var(A)/Var(P). Where Var(A) is additive variance in this case family variance and where Var(P) is phenotype and the sum of all variance i.e. family variance added together with residual variance.

Due to the fact that damage caused *H. fraxineus* in the form of stem necrosis is not visible until the following season, analysis of variance (ANOVA) of the population planted in Snogeholm was not performed in this study.

Results

Alnarp progeny trial

Bud burst:

Assessments of bud burst were conducted 4th May 2015 (Figure 6) and 21st of May in 2016 (Figure 7). Bud burst in May 2015, varied among seedlings within families, particularly for families 121 and 122 (Figure 6) all stages of budburst existed. The variance within families (91%) was larger than among families (9%). There was no significant difference between plant types (p=0.1961).

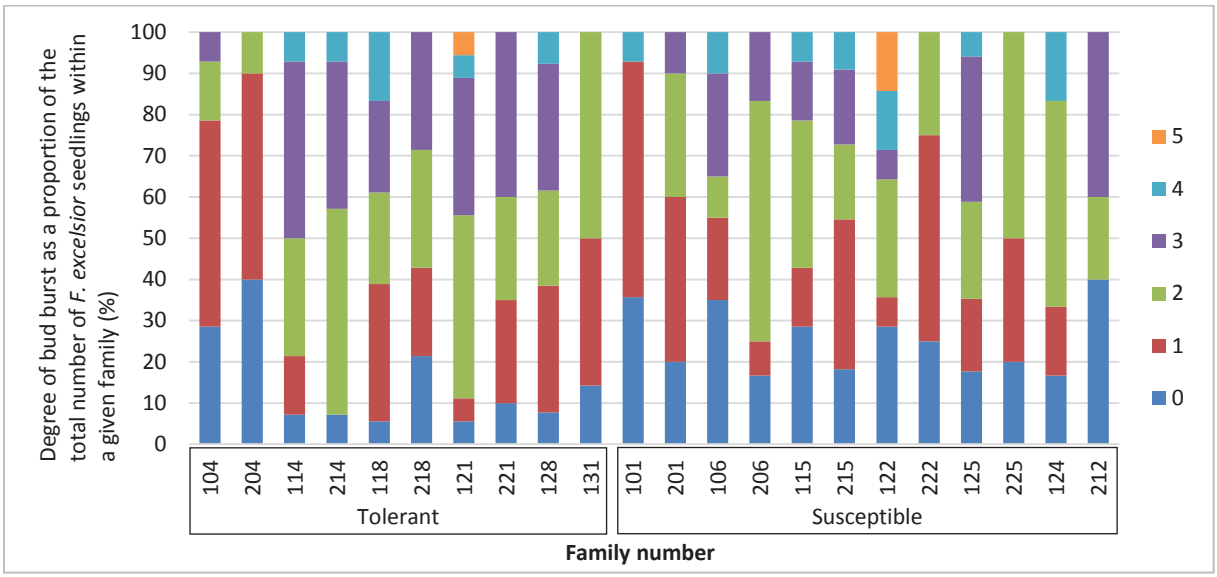

Figure 6. The degree of bud burst for each family at the first assessment, 4th of May 2015, rated into 6 different bud burst categories, whereby 0 = the bud is still dormant, 1 = the bud is green and have begun to swell, 2 = the leaves have begun to emerge from the bud, 3 = the leaves have begun to open and petioles are visible, 4 = the leaflets are open and petioles have begun to extend and 5 = the leaves area fully opened and expanded (Table 2). Seedlings within a family were combined to visualize the proportion representing different stages of bud burst within a given family.

In 2016, most of the seedlings had leaves fully opened and expanded (Figure 7). In families 212 and 222, all individuals had fully burst. Seven families had individuals representing different stages of bud burst (family numbers: 104, 204, 118, 218, 201, 115, and 225). However, 92% of the variance mainly depended on the individuals within families and less on the variance among families (8% of the variance). There was no significant difference between plant types (p=0.0848), but there was a significant difference (p <0.0001) for blocks indicating that bud burst was slightly dependent on which block they were planted in. For example, blocks 6, 9 and 10 had a lower mean rating for bud burst than the remaining blocks. The mean rating for blocks 9 and 10 was 4.24 and the mean rating for block 6 was 4.48. For the remaining blocks, the mean rating for bud burst ranged between 4.67 and 4.85.

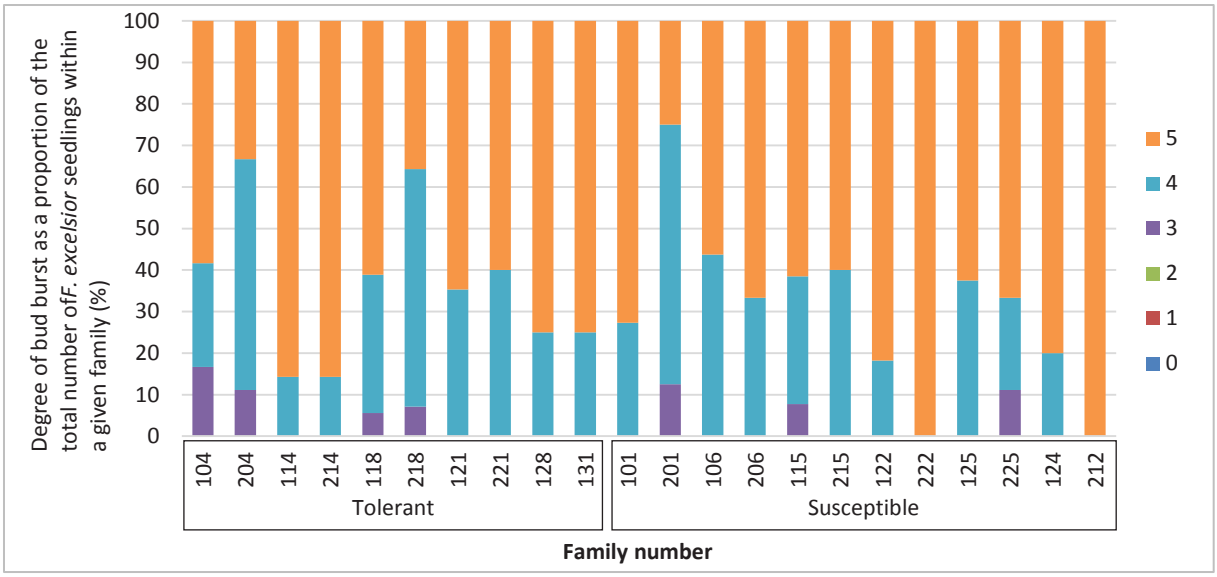

Figure 7. The degree of bud burst for each family at the first assessment, 21st of May 2016, rated into 6 different bud burst categories, whereby 0 = the bud is still dormant, 1 = the bud is green and have begun to swell, 2 = the leaves have begun to emerge from the bud, 3 = the leaves have begun to open and petioles are visible, 4 = the leaflets are open and petioles have begun to extend and 5 = the leaves area fully opened and expanded (Table 2). Seedlings within a family were combined to visualize the proportion representing different stages of bud burst within a given family.

Survival:

Seedling survival in Alnarp was calculated on data from health assessments in September 2014 and September 2016 (Figure 8). Already in the first year, 2014, mortality due to non-pathogen induced factors was 5%. By 2016, cumulative mortality was 13%, the increase being pathogen induced. Susceptible families 101, 122 and 206, had the lowest survival (<80%) at the last assessment. For seven families (114, 214, 118, 218, 221, 222, and 124), survival remained constant and for six families, survival was 100%. The families ranked as being tolerant to the disease had, in total, higher survival (93%) than susceptible families (83%).

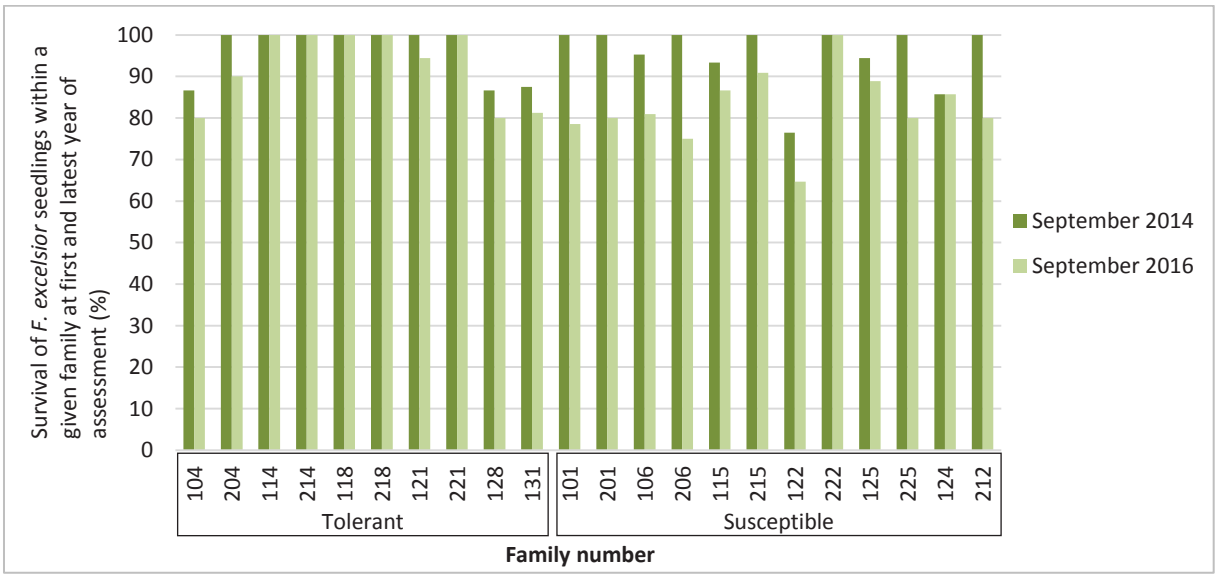

Figure 8. Survival of seedlings calculated as proportion of total number of seedlings within a given family.

Health class rating (HCR):

Assessments of health status was conducted in 2015 during May and July, and in 2016 during June, July and September. The health class rating in July 2015 (Figure 9), showed large variation within and among families; the variance was highest among individuals within families (88%) and lower among families (12%). The families with higher susceptibility status based on the previous assessments (Stener, 2013) had a higher mean HCR than those rated as being more tolerant. There were significant differences between the mean rating for tolerant and susceptible seedlings, (p=0,00125); the mean HCR for susceptible families was 2.62 compared to 2.01 for tolerant families (Figure 9). Nearly all susceptible families had seedlings with HCR 5, while only three out of 10 families denoted as being tolerant had seedlings with HCR 5 rating. There were significant differences (p= 0,0274) between the two plant types (1-year-old plants grown in Uppsala and 2-year-old plants grown in Ekebo), whereby plant type Uppsala had a lower average HCR (2.17) than the Ekebo plant type (2.48).

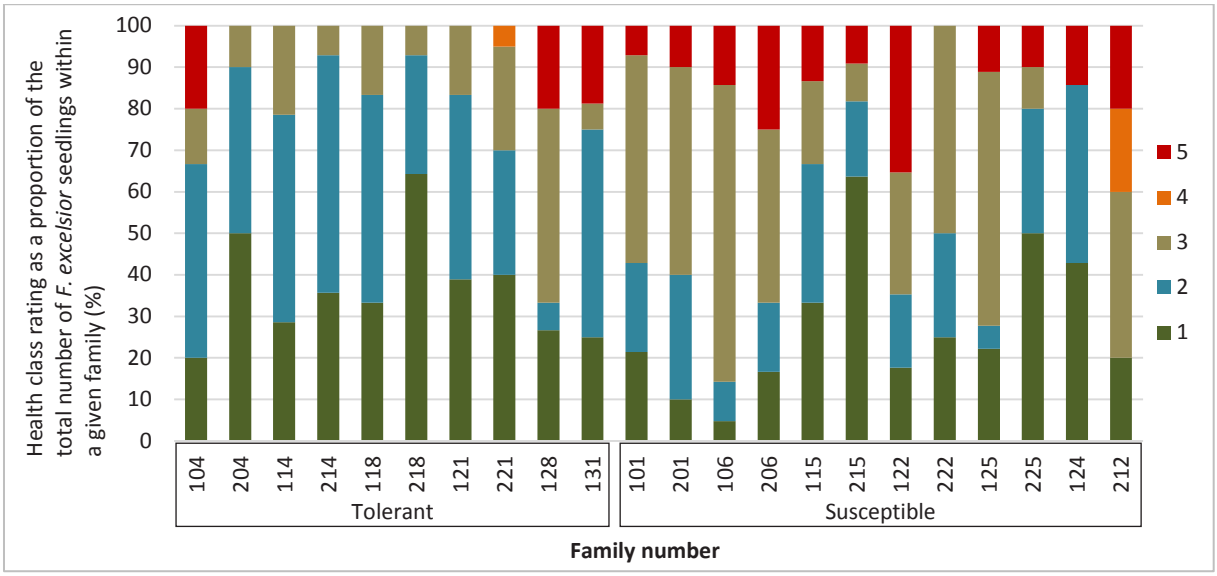

Figure 9. Health class rating (HCR) denoting the degree of ash dieback symptoms in July 2015, whereby 1 = symptomless, 2 = slightly damaged, 3 = moderately damaged, 4 = severely damaged and 5 = dead (Table 3). Seedlings within families were combined to visualize the proportions of HCR ratings within a family.

The health assessment in July 2016 (Figure 10) also displayed large variation within and among families; 87% of the variance was found within families and 13% among families, and heritability (h^2) = 0.54. The mortality was higher for susceptible families compared to those rated as being more tolerant (19% and 7%, respectively). The average health class rating for susceptible seedlings remained higher than for tolerant seedlings (HCR 2.60 for susceptible and 1.91 for tolerant), and the differences between them also remained significant (p<0.001). During this assessment, there was no significant difference between plant types (p=0.0538).

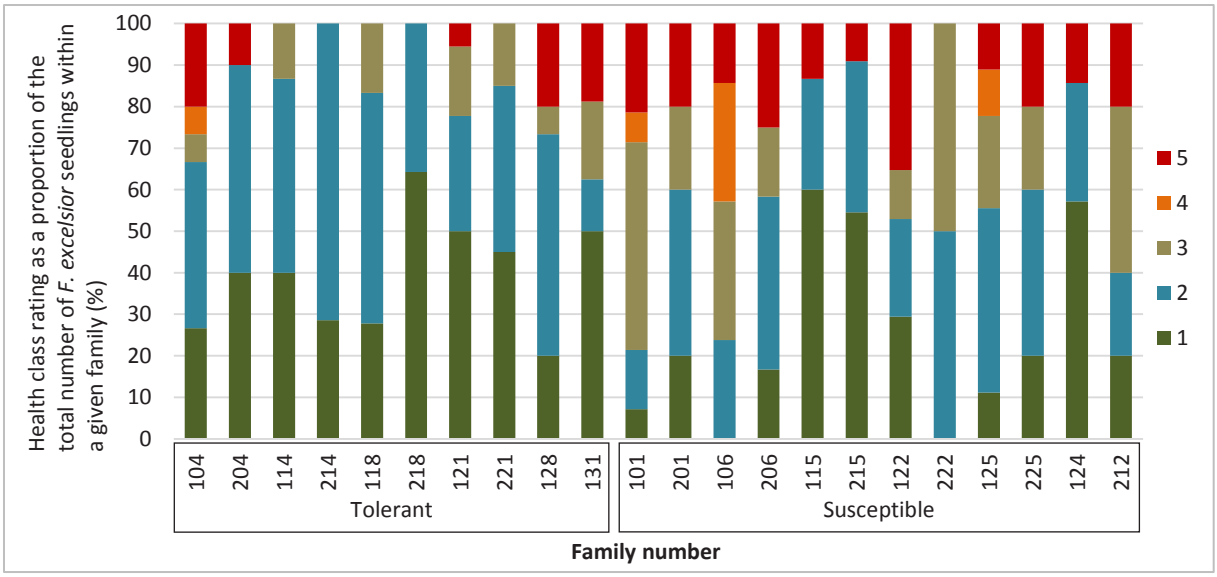

Figure 10. Health class rating (HCR) denoting the degree of ash dieback symptoms in July 2016, whereby 1 = symptomless, 2 = slightly damaged, 3 = moderately damaged, 4 = severely damaged and 5 = dead (Table 3). Seedlings within families were combined to visualize the proportions of HCR ratings within a family.

In September 2016 (Figure 11), large variance in HCR was detected both within and among families; 85% of the variance was found within families and 15% was found among families, and h^2=0.61. Nearly all seedlings having HCR 1 in July 2016 progressed to HCR 2 by September. Exceptions were found for tolerant families 204 and 131 whereby some seedlings remained symptomless (HCR 1) throughout the season, including at the last assessment in September (Figure 11). All seedlings in tolerant family 214 and nearly all in tolerant family 218, were rated as HCR 2. This is in large contrast to susceptible families whereby none were rated in the lowest HCR (HCR 1), and with the exception of few, most had higher average HCR than tolerant families (Figure 11).

The highest mortality was found in family 122 both at the assessment in July 2015 and in September 2016 whereby 35% of the seedlings had died. No seedlings had died in the corresponding family 222, and only a small, but significant difference between plant types (p=0,0276) was detected; HCR for Ekebo plants was 2.85 and for Uppsala plants, 2.59. Seedling mortality increased in total for the more susceptible families, from 18% in 2015 to 19% in 2016 but mortality did not change for tolerant families. By the end of third year assessment, the mean HCR for susceptible families remained higher (HCR 3.01), than for tolerant families (HCR 2.40) and the differences between them were still significant (p<0.001).

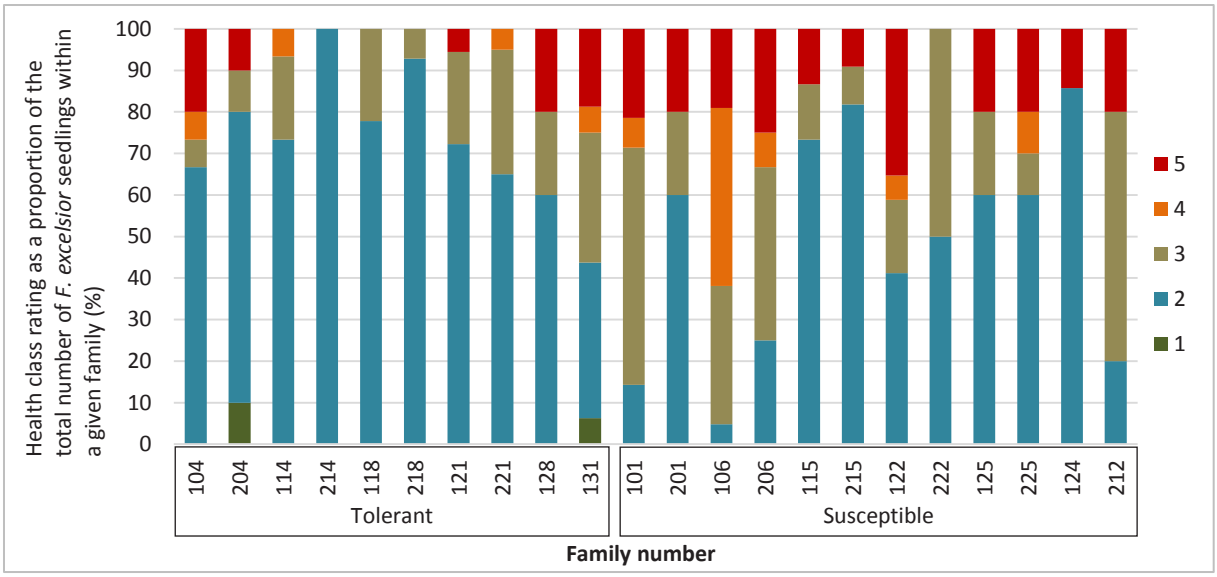

Figure 11. Health class rating (HCR) denoting the degree of ash dieback symptoms in September 2016, whereby 1 = symptomless, 2 = slightly damaged, 3 = moderately damaged, 4 = severely damaged and 5 = dead (Table 3). Seedlings within families were combined to visualize the proportions of HCR ratings within a family.

Stem form:

The assessment of stem form was conducted in October 2015 and September 2016. In October 2015, the seedlings mainly had a single stem (Figure 12) and the differences between tolerant and susceptible families were minimal; 70% of susceptible seedlings and 78% of tolerant seedlings had stem form rating 1. However, 14 of 22 families (7 tolerant and 7 susceptible) had seedlings with stem form rating 3 indicating a 'bushy' stem form. For family 212, half of the seedlings had three or more stems, whereby more than 30% of trees in three other susceptible families (106, 122, and 125) had similar rating for stem form. While the incidence of bushiness indicated by a stem form rating of 3 occurred in both susceptible and tolerant families, the proportion of trees with this rating was markedly higher for susceptible families (16%) than for tolerant families (9%). The variance nearly only depended on the seedlings within families (98%). Significant differences in stem form ratings were found between the two plant types ($p=0.0123$) whereby the mean rating for plant type Ekebo was 1,46 and 1,26 for plant type Uppsala. The mean number of stems, for individual seedlings, was therefore also higher for plant type Ekebo 1.69 compared with 1.42 for plant type Uppsala. Significant differences were also found among blocks ($p=<0.0001$). Blocks 3, 8 and 9 had a higher mean rating for stem form at 1.8, 1.94, and 1.75, respectively, compared to the remaining blocks (stem form rating ranged between 1.18 and 1.54).

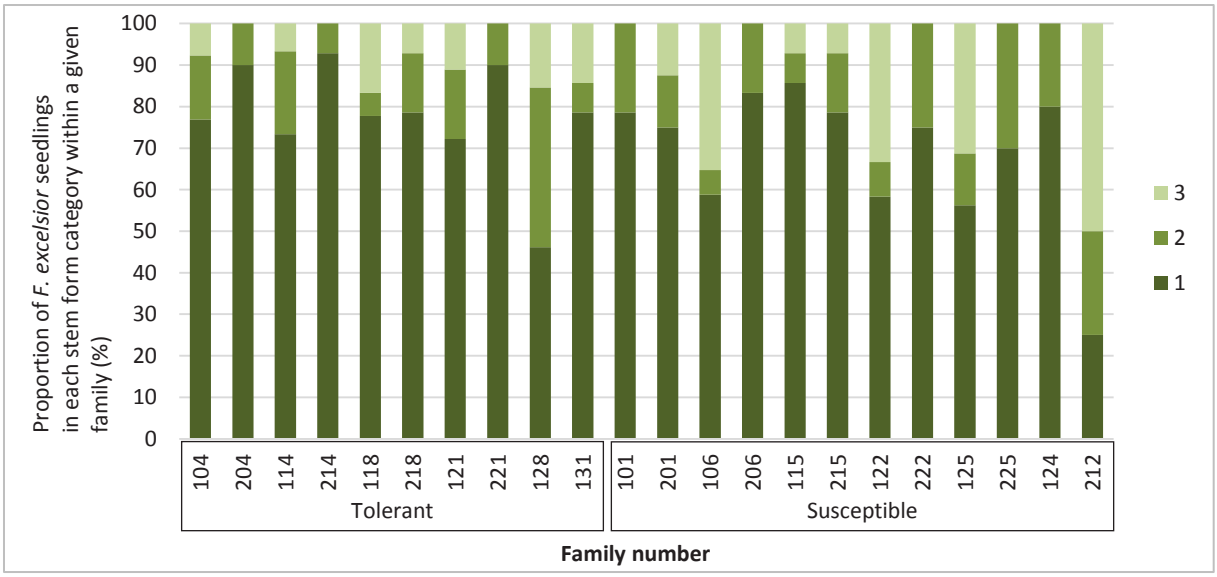

Figure 12. Stem form, October 2015, rated into three different categories depending on the number of stems, 1 = one single stem, 2 = one or two new stems have sprouted and 3 = three or more stems have sprouted (Table 4). Seedlings within families was combined to visualize the proportions of different ratings within a given family.

In September 2016, large variation in stem form was found within families (85%) and among families (15%) (Figure 13). Significant differences ($p < 0.0001$) were found for plant types, whereby Ekebo plants had a higher mean rating (1.86) than Uppsala plants (1.48). The mean number of stems were consequently higher for plant type Ekebo compared with plant type Uppsala, 2.15 and 1.7, respectively. For the tolerant families, 55% of the total number of seedlings had a single stem, compared with 31% of the total number of susceptible seedlings. In the highest category, which comprises three or more stems, 27% of the susceptible seedlings were represented compared to only 6% of the tolerant seedlings. The mean rating for susceptible families was 1.96 and for tolerant families 1.51 and the mean number of stems, for individual seedlings, were 2.64 for known susceptible seedlings and 1.78 for known tolerant seedlings.

For family number 214, nearly all seedlings had stem form rating 1 (Figure 13); which was lower for its corresponding family 114, where rating 1 and 2 had the same proportion of seedlings (approximately 47% each). For families 201 and 225, most of the seedlings were rated to category 1 (approximately 67% and 75%, respectively), while their corresponding families 101 and 125 had less seedlings rated to category 1 (approximately 27% for family 101 and 19% for family 125). Unlike their corresponding families 201 and 225, families 101 and 125 had seedlings with stem form ratings in all three categories. Family 106, a known susceptible family, had no seedlings with a single stem and the majority had three or more stems.

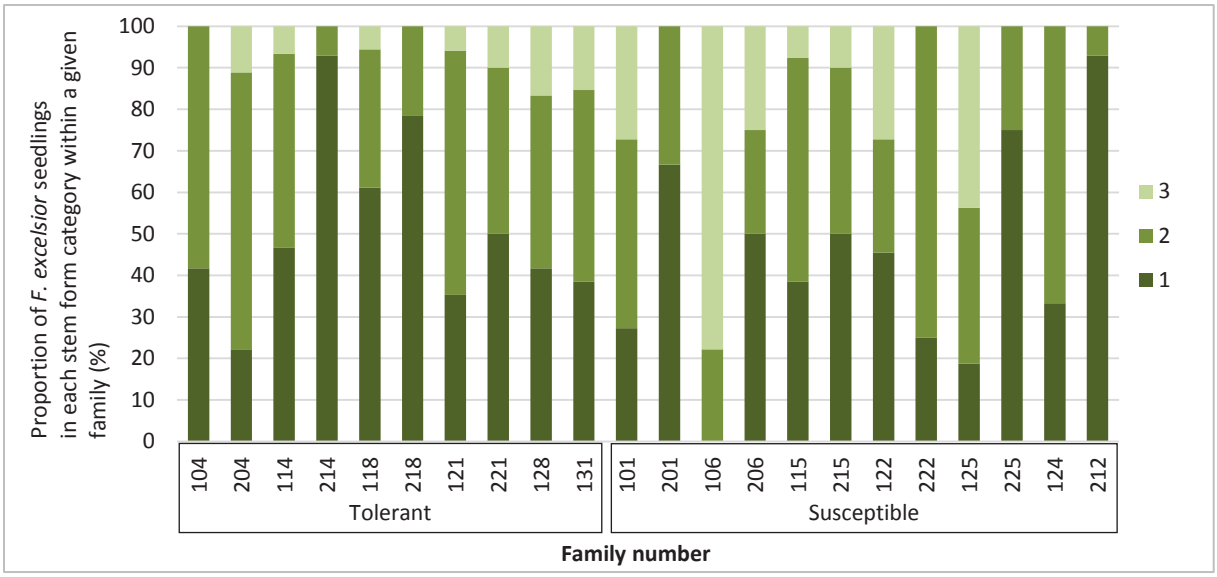

Figure 13. Stem form, September 2016, rated into three different categories depending on the number of stems, 1=one single stem, 2 = one or two new stems have sprouted and 3=three or more stems have sprouted (Table 4). Seedlings within families was combined to visualize the proportions of different ratings within a given family.

An overview of the measured traits (bud burst, survival, health class and stem form) for each family is presented in Table 6.

Table 6. Compilation of mean values for the different ratings (bud burst, health class and stem form) for each family and plant type. Bud burst rating was conducted in May 2015 and 2016, calculations of survival was based on data collected in July 2015 and July 2016, mean values for health class rating was based on assessments conducted in July 2015 and in July 2016. Calculations of mean stem form rating was based on data collected in October 2015 and September 2016.

Family number	**Plant type**	**Trait**							
		Mean bud burst rating		**Survival (%)**		**Mean health class rating**		**Mean stem form rating**	
		2015	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
101	Ekebo	0.86	4.73	92.86	78.57	2.50	3.14	1.21	2
201	Uppsala	1.30	4.13	90	90	2.70	2.50	1.38	1.75
104	Ekebo	1.00	4.42	86.67	73.33	2.53	2.43	1.31	1.58
204	Uppsala	0,7	4.22	100	90	1.60	1.85	1.20	1.33
106	Ekebo	1.55	4.56	85.71	85.71	3.10	3.33	1.76	2.78
206	Uppsala	1.75	4.67	75	75	3	2.78	1.17	1.89
212	Uppsala	1.60	5.00	100	80	3.20	2.78	2.25	1.75
114	Ekebo	2.29	4.86	100	100	1.93	1.77	1.33	1.60
214	Uppsala	2.36	4.86	100	100	1.71	1.68	1.07	1.07
115	Ekebo	1.57	4.50	86.67	86.67	2.27	1.76	1.21	1.69
215	Uppsala	1.64	4.60	90.91	90.91	1.73	1.68	1.30	1.60
118	Ekebo	2.11	4.56	100	100	1.83	1.87	1.39	1.44
218	Uppsala	1.64	4.29	100	100	1.43	1.35	1.29	1.21
121	Ekebo	2.44	4.65	100	94.44	1.78	1.82	1.39	1.71
221	Uppsala	1.95	4.60	100	100	1.95	1.67	1.10	1.60
122	Ekebo	2.14	4.82	64.71	64.71	3.18	2.86	1.75	1.82
222	Uppsala	1.00	5.00	100	100	2.25	2.29	1.25	1.75
124	Ekebo	1.83	4.80	85.71	85.71	2	1.79	1.20	1.67
125	Ekebo	1.94	4.63	88.89	88.89	2.72	2.64	1.75	2.25
225	Uppsala	1.30	4.56	90	80	1.90	2.55	1.30	1.25
128	Ekebo	2.00	4.75	80	80	2.80	2.43	1.69	1.75
131	Ekebo	1.36	4.75	87.50	81.25	2.38	2.22	1.36	1.77
Family variance (%)		9	8	-	-	12	13	2	15
Plant type significance level		0.1961	0.0848	-	-	0.0274	0.0538	0.0123	<.0001

Snogeholm

Survival:

The ash clones at Snogeholm trial established in May 2016 showed high survival throughout the first season; 98.5% of the total number of seedlings planted survived (Figure 14). Some ramets of certain clones had died before the first assessment in July 2016 (clones 8, 3001, 3003, 3005, 3011 and 3056) but then survival remained the same for those clones in subsequent assessments. For other clones (3010, 3011, 3020, 3037 and 3051) however, survival decreased (by up to 8% within a clone) between July and September.

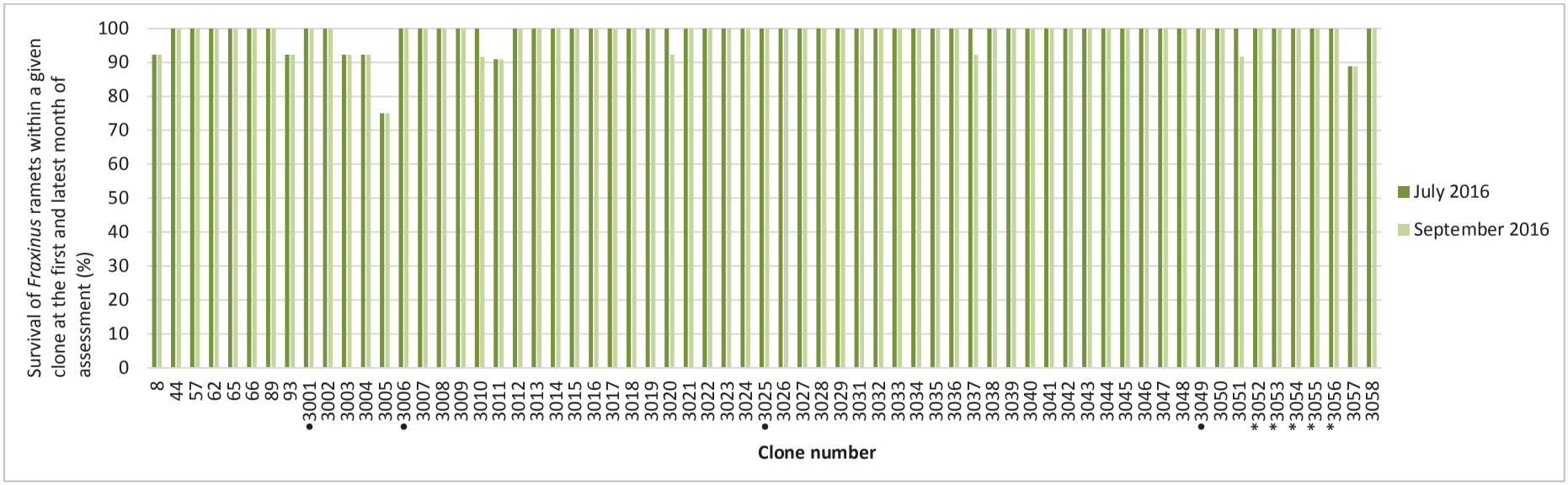

*Figure 14. Survival of ramets calculated as proportion of total number of ramets in each clone, where • = known susceptible clones and * = Asian Fraxinus species.*

Disease incidence:

At the health assessment in July, 21 of 65 clones did not show any symptoms of ash dieback. In September though, that number changed. All clones had developed symptoms, and for three clones in particular (3001, 3016 and 3019), all ramets showed symptoms. Of the 65 clones included in this trial, 52 had more than half of their ramets showing disease symptoms by September 2016. For the remaining 13 clones (57, 3011, 3013, 3017, 3020, 3021, 3026, 3041, 3044, 3051, 3055, 3056 and 3057) the large majority of their ramets were still symptomless, two of the clones were Asian *Fraxinus* species (3055 and 3056) and the rest were of tolerant origin. In July, 17% of the Asian clones showed early symptoms of leaf necrosis and in September that proportion increased to 61%. This reflects the proportion accounted for all clones included in the trial, whereby 14% of all clones showed symptoms in July and 69% in September.

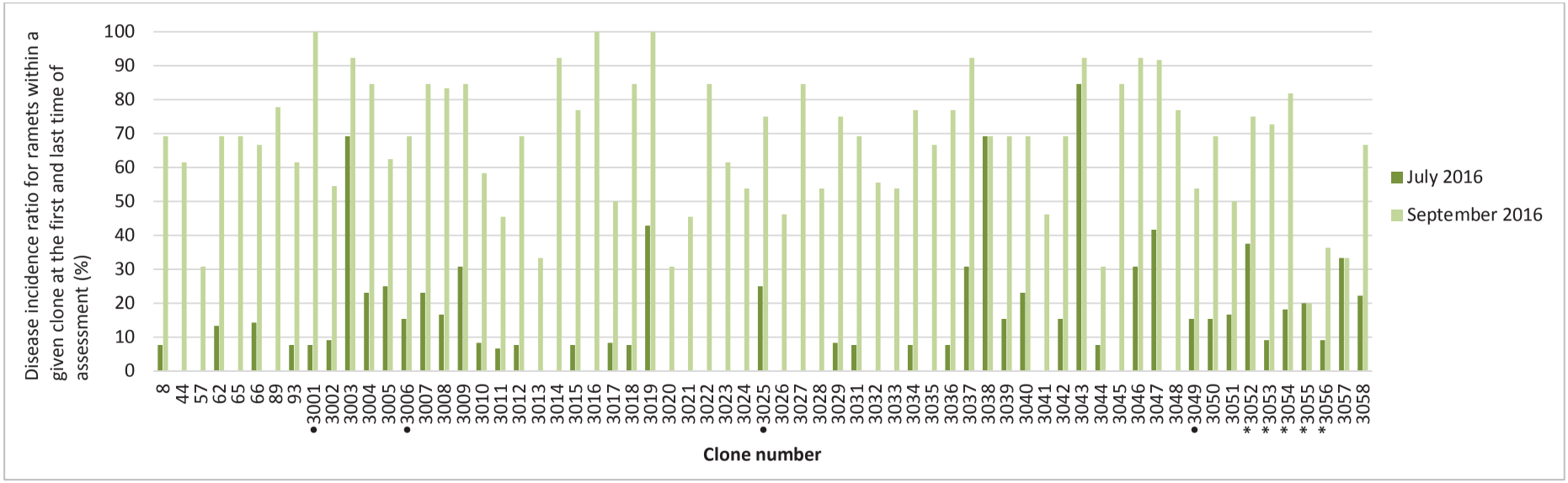

*Figure 15. The symptom development for all clones in July and September calculated as a proportion of total number of ramets within a clone, where • = known susceptible clones and * = Asian Fraxinus species.*

Health class rating (HCR):

Assessments of health status at the trial in Snogeholm were conducted in July and September 2016. At the first assessment most of the ramets were symptomless, but some clones had ramets showing symptoms of infection (small necrosis on leaves). Clones 3043, 3038 and 3003, had more than half of their ramets showing early symptoms equal to HCR 2. Some clones had dead ramets (8, 93, 3003, 3004, 3005, 3011, and 3057), and two clones (3010 and 3037) had ramets showing symptoms equal to HCR 4, all clones were known to be tolerant. None of the Asian clones went unaffected, still HCR 1 was the dominating category (3052: 63%, 3053: 91%, 3054: 82%, 3055: 80%, 3056: 91% of the ramtes in HCR 1). The susceptible clones (3001, 3006, 3025 and 3049) all showed high number of symptomless ramets and in general health class rating did not differ much between known susceptible and tolerant clones at this point.

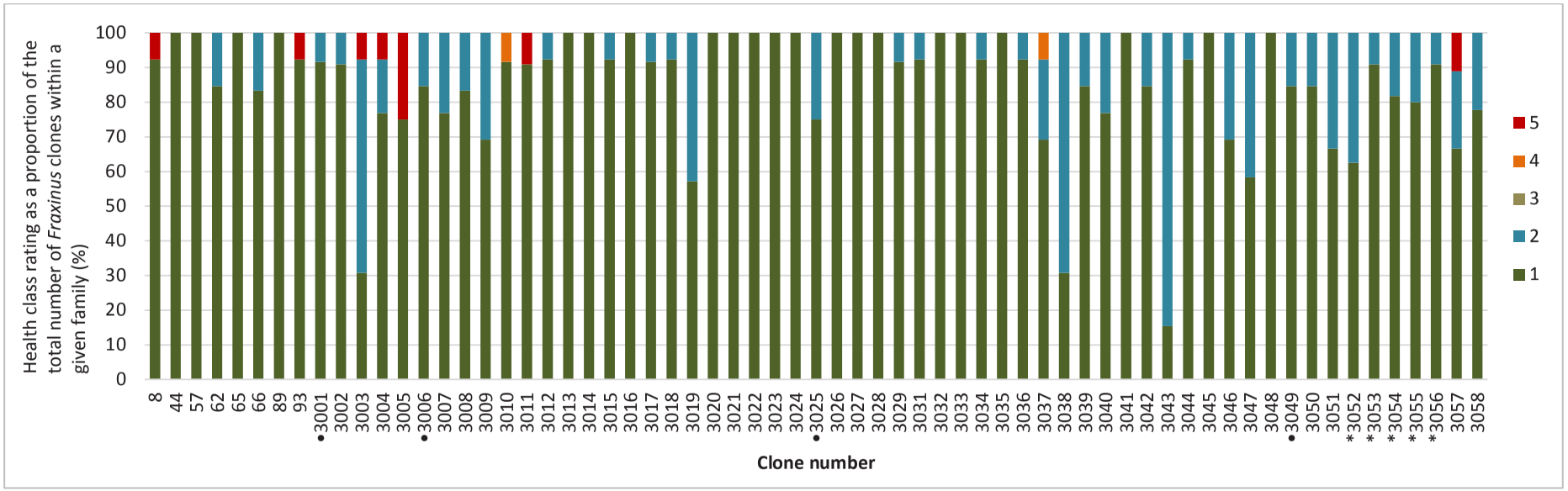

*Figure 16. Mean health class rating (HCR), July 2016, of clones given expressed as a proportion of the total number of ramets within a clone showing symptoms according to the given health class rating of increasing disease symptoms, whereby 1 = symptomless, 2 = slightly damaged, 3 = moderately damaged, 4 = severely damaged and 5 = dead (Table 3). Where • = known susceptible clones and * = Asian Fraxinus species.*

At the last assessment (Figure 17) there were no clones without symptoms, most of the clones had ramets with HCR 2 and all ramets in clones 3001 (a known susceptible), 3016, 3019 and 3024 were rated to HCR 2. The second most frequent rating was HCR 1 and in some cases HCR 1 was the dominant rating within a clone (57, 3011, 3013, 3020, 3021, 3026, 3041, 3044, 3055, 3056 and 3057). The number of clones with dead ramets had increased from the first health assessment, in July seven clones experienced mortality and in September that number increased to 11 clones (8, 93, 3003, 3004, 3005, 3010, 3011, 3020, 3037, 3051 and 3057) none of them of susceptible origin. For these 11 clones, mortality ranged between 8-25%. Clone 3056, one of the Asian *Fraxinus* species, had a ramet with HCR 4. Few clones (66, 3008, 3021, 3025, 3042, 3047 and 3052) had ramets which showed symptoms equivalent to HCR 3, one of which (3025) was a known susceptible and one an Asian *Fraxinus* species (3052).

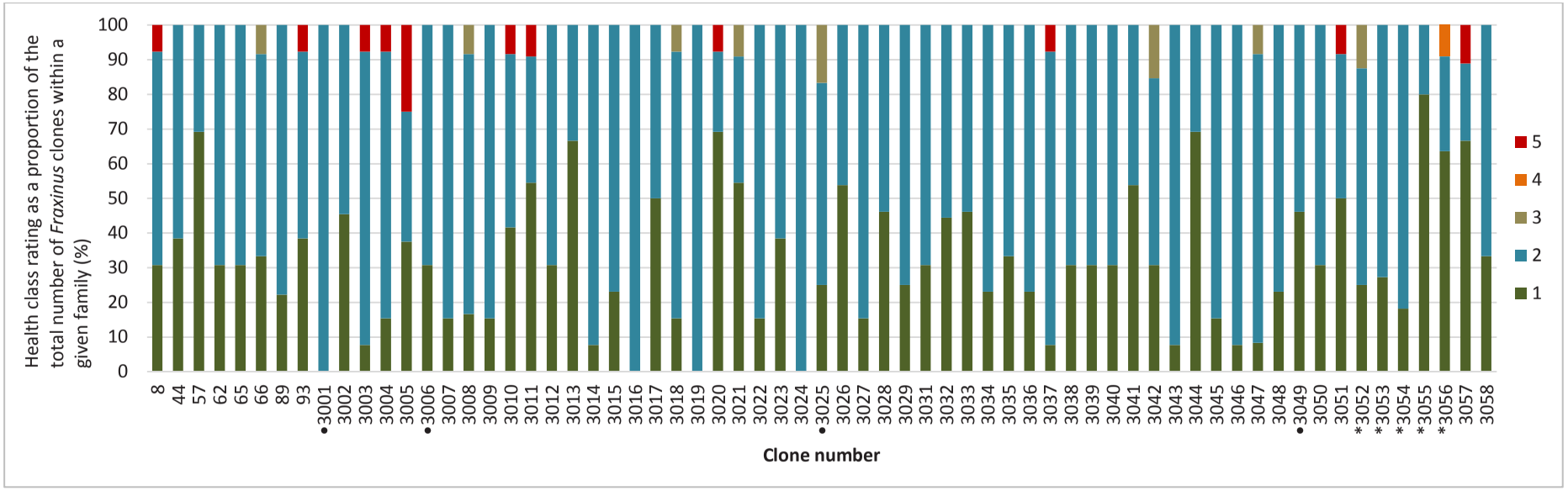

*Figure 17. Mean health class rating (HCR), September 2016, of clones given expressed as a proportion of the total number of ramets within a clone showing symptoms according to the given health class rating of increasing disease symptoms, whereby 1 = symptomless, 2 = slightly damaged, 3 = moderately damaged, 4 = severely damaged and 5 = dead (Table 3). Where • = known susceptible clones and * = Asian Fraxinus species.*

Discussion

Alnarp progeny trial

The study provides an early indication of growth performance and tolerance to dieback damage under chronic infection pressure by *H. fraxineus*. The results presented will therefore be of importance for further studies on the subject servingas a baseline for comparison with future assessments of genotype performance in order to prioritize selection of superior (i.e. disease tolerant) ash clones. Consequently, the progeny trial enables us to identify, preserve and maintain specific healthy genotypes of interest, which can be of importance for ecosystems and the future survival of many ash associated species in forests and urban and rural landscapes.

Survival:
Already the same year as the seedlings were planted, 5% of the seedlings had died. This indicates that a share of the mortality of seedlings was due to establishment causes, e.g. problems with rooting, microclimate effects, or competition with vegetation, and not due to ash dieback. The cumulative mortality in 2016 reached 13%, resulting in a mortality of approximately 4% per year since its establishment in spring 2014. In a previous study performed by Stener (2013), albeit on slightly older material, the mortality in Snogeholm and Trolleholm was approximately 7% per year. In a progeny trial in Lithuania (Pliura et al. 2011) the survival only reached 10% when the trees were eight years old. The seedlings in the current study were either 1-year-old or 2-year-old plants making them younger than the trees in comparable studies (Stener 2013, Pliura et al. 2011), which might explain differences in survival. Another reason could be the selection of material; since the previous studies cited had not done a direct selection for tolerant trees. The material established in the Alnarp trial was selected for its known susceptibility (high or low), creating a possibility to increase the survival at least for those progenies originating from tolerant mother clones in the seed orchards. As such, the survival in 2016 was higher for tolerant families (mean survival of 93%) compared to susceptible families (83%), and a large proportion (71%) of plants killed during the first years belonged to known susceptible mother clones. Family 122, had the highest mortality in 2016 with 35 % of its seedlings that had died, though already in 2014, the mortality was high (24%) due to establishment causes. None of the selected tolerant families had a survival less than 80%. The higher survival for these established tolerant clones at this young age is promising for its future performance in light of chronic infections of *H. fraxineus*.

Cumulative mortality increased from 11% in 2015 to 13% in 2016. This increase was smaller than between 2014 and 2015 when the mortality went from 5% to 10%. The number of dead seedlings in 2016 was therefore fewer than in the previous years. An increase in mortality is expected over time, but what is remarkable is that seedlings went from a low health class rating to the highest health class rating (i.e. dead), within a year. This was the case for one seedling in susceptible family 201, which went from HCR 1 to HCR 5, and for seven seedlings (four of them from susceptible families 101, 106 and 225 and three from tolerant families 121, 128 and 204), whose health class status was elevated from HCR 3 to HCR 5 within a single season. The development of the disease can obviously be extremely fast especially on younger seedlings. The lower survival in smaller or younger seedlings compared to larger trees may be attributable to the fact that the lesions which form can more easily girdle a small branch or stem (Cleary et al. 2013, McKinney et al. 2014, Kirisits et al. 2012, Witzell et

al. 2014). Nearly all seedlings that had undergone a dramatic change in HCR within a single season were shorter than the average height of all seedlings, with the exception of two seedlings. Thus size may not be the only attributable reason for rapid expansion of the disease. Another plausible explanation could be that the internal expansion of lesions was much larger than what was visible in the bark, a known phenomenon (Marčiulynienė et al. 2017, Cleary et al. 2013, McKinney et al. 2012, Gross et al. 2014), causing more damage than what was apparent at the assessment in July 2015.

Health class rating (HCR):

For all health status assessments, the variance was greatest within families, indicating a large variance among seedlings regardless of family affiliation. Variance among families was smaller but still a relevant factor. The narrow sense heritability that was calculated ranged between 0.48 and 0.61, for the three assessment periods (July 2015, July 2016 and September 2016). The value of h^2 in this study is relatively insecure, since it was few families included in the trial but also since it was not possible to calculate standard error (SE) with the information given from R. A high SE is expected since the trial is based on few families. However, h^2 is still comparable and a h^2 ranging between 0.48 and 0.61 is considered as a high value for assessing particular traits, and indicates that the damage caused by the disease (i.e. the degree of susceptibility) is strongly genetically controlled. Previous studies (Kjær et al. 2011, Pliura et al. 2011, Stener 2013) also showed high h^2 values for damage traits ranging between 0.37 and 0.52, suggesting that the degree of susceptibility is a heritable trait that is genetically controlled.

Susceptible families had a higher mean HCR and correspondingly, higher mortality compared to tolerant families. Several studies indicate large genotypic variation in susceptibility (Pliura et al. 2011, 2014, McKinney et al. 2011, Kjær et al. 2011, Kirisits et al. 2011, Stener 2013). In all of these studies no trees went completely without infection and the development of symptoms, but disease severity differed substantially among genotypes. Similarly, in this trial, nearly all seedlings were affected by the disease but disease severity varied immensely. The fact that also some progenies originating from tolerant mother clones showed high disease severity may be attributable to the fact that they originated from open pollination. Thus, the susceptibility status is only known for the mother, while the information about the male parent is unknown. Pollen from a susceptible father clone may likely exhibit high susceptibility among the offspring as well.

Dieback symptoms on shoots, twigs and branches of seedlings became evident the year after trial establishment. By July 2015 it was obvious that 193 of 284 seedlings showed some level of infection (i.e. disease incidence was 68%). However, the severity of the disease (as denoted by HCR) was more pronounced on susceptible clones than on tolerant clones. This corresponds with the earlier studies performed (McKinney et al. 2011, Kjær et al. 2011 and Pliura et al. 2014). Despite the small test population, it is obvious that some few genotypes are superior in their performance over others. For example, families 214 and 218 displayed lower mean HCR during years 2015 and 2016 compared to others. Similar to Pliura et al. (2014) which involved assessments of young progenies, there has been a considerable increase in disease incidence and severity on all seedlings since trial establishment, but the variation among families remained consistent between years. By the end of 2016 only two seedlings had the lowest rating (HCR 1), whereas earlier in July, the number of unaffected seedlings (i.e. with HCR 1) was considerably higher (n=96). The sporulation period is normally from June to September, with the peak happening usually between July and mid-August. It is likely given the increase in HCR between this period that a great change in the infection rate and obvious disease

expression occurred between the July and September assessment. However, the large number of seedlings that went from HCR 1 to HCR 2 during the season was also a reflection of the too wide criteria set for HCR 2. Part of the criteria ranged from brown necrosis on leaves and rachises to wilted leaves which fits the scenario for most seedlings at that later assessment, however despite this some were more or less affected by the disease. A recommendation based on this early work would be to expand the HCR classification with an additional category, making it possible to categorize seedling damage more specifically. Therefore, a suggestion would be to divide HCR 2 into two separate categories based on how big proportion of the seedling and its foliage is showing symptoms. Being able to differentiate seedlings and their disease severity, more specifically, could be especially important for assessing seedlings of this young age (less than 5-years-old) and would probably also result in a clearer distinction of damage among seedlings.

Contrary to the notion that disease incidence and severity will increase over time, some 13 seedlings, actually underwent a reduction in HCR. Ten seedlings went from HCR 4 to HCR 3 and three seedlings from HCR 4 to HCR 2, within an individual season. Reasons for 'improvement' in health status may be due to the tree's natural reaction when it becomes infected by *H. fraxineus*, namely the formation of epicormic shoots, but also due the normal growth that occurs during the season. The criteria for HCR 4 includes seedlings with four or more necrotic lesions or 50% of the seedling is damaged. In the beginning of the season, seedlings may appear more severely damaged than at the end of the season if one takes into account that the normal growth of the tree exceeds that of any necrosis expanding in a stem lesion, and that epicormics have formed as replacement shoots for those damaged by the fungus. The cumulative damage on a seedling will hence be lower if it is able to compensate and continue growing normally throughout the season, hence exhibiting overall lower disease severity. Similar observations were shown by Kirisits et al. (2011).

The formation of epicormics is a stress response induced when the tree get infected by *H. fraxineus* whereby new shoots sprout either from the proximal end of a necrotic lesion on a branch which has been killed by the fungus or, in the case of younger trees, from the lower part of the original stem to compensate for lost tissue (McKinney et al. 2014, Cleary et al. 2013). A seedling that may have been rated as being severely diseased during one season may have been classified at a lower HCR during the following season due to epicormics that have sprouted. For two seedlings in this trial, the original stem was recorded as either dead or missing in 2015, but it was obvious that in 2016 new stems sprouted from the base of the seedling which resulted in lower HCR (HCR 2) for both seedlings at the last assessment in 2016. It might be possible to weight the ratings in a way that if a seedling has had a dead stem it cannot receive a low rating, since a result like this might complicate further selection of seedlings from this trial. Nevertheless, the formation of epicormics should be factored into assessments as they may cause disease severity ratings to drastically change within and among years.

Another factor affecting the classification of damage during health assessments is the time of year when infections become visible. Infections that occur on leaves and rachises during the current growing season will not become evident as necrosis on the stem until in the following season, since it takes time for the fungus to spread and develop stem cankers (Bengtsson et al. 2014). Thus, the internal spread of the fungus during the growing season is not at all reflected in the HCR, and usually gives lower ratings during the current season compared to the following year. For 12 seedlings, a rather large change in HCR occurred from the last assessment in 2015 to the first assessment in 2016. Four seedlings went from HCR 1 to HCR 3, three seedlings went from HCR 1 to HCR 4, one seedling

went from HCR 1 to HCR 5 and four seedlings went from HCR 2 to HCR 4. These large increases in disease severity may be attributable to the internal spread of the fungus being several times larger than what might be visible on the bark surface (Marčiulynienė et al. 2017, Cleary et al. 2013, McKinney et al. 2012, Gross et al. 2014) and the fact that the growth of the fungus may continue to spread even during plant dormancy. However, disease severity also depends on factors such as climate, the inherent virulence of the pathogen and tree vigour; three critical elements of the disease triangle (Manion 1991). The interaction among these three factors could largely affect the seedlings and cause drastic changes in HCR both within and between seasons. If conditions (temperature, humidity) are favourable to *H. fraxineus,* the sporulation period may start earlier and extend longer in the season (Timmerman et al. 2011, Gross et al. 2014), giving an advantage to the pathogen for a longer infection period. Indeed, it has been observed that fully mature and ripe apothecia can be formed in southern Sweden as early as the end of May (M. Cleary, pers. obs.). But infection of a tree also depends on its vitality and host genetics which by themselves are also affected by climate. If the environment, climate, is unfavourable for tree growth, but still adequate for the pathogen, trees may be more vulnerable to attack.

For three seedlings, it was not possible to detect the necrotic lesions in September that were earlier identified in July. However, this did not affect their HCR but given that, a recommendation would be to mark the necrotic lesions with an indelible pen to make it easier to find them during subsequent assessments to track the expansion of necrosis throughout a season. However, even marking lesions may not guarantee that they may be located at a later date since Kirisits et al. (2011) found that dead twigs and branches naturally fall off the tree and are then no longer included in the health assessment. This scenario could therefore give the impression of a healthier tree than what may in fact be the case.

Stem form:

It became obvious during the second and third year assessments that stem form varied substantially among individuals and that a criteria for rating stem form would be especially important considering not only resistance traits but also quality traits important for future tree breeding efforts. The assessment of stem form indicated what type of shape the trees exhibited. In 2015, there was no difference among families in stem form and most of the seedlings had only single stems, regardless of their susceptibility status. As the first assessments were done the year after establishment the development of the disease was not fully expressed until the second year when large necroses on the main stem which had subsequently died back then resprouted the following season. At that time large variation was detected among tolerant and susceptible families whereby more than half of the tolerant seedlings still had a single stem, compared to about one third of the susceptible seedlings; a direct consequence of the more severe disease severity exhibited by those seedlings. Thus, tolerant families have a tendency to give a much less 'bushy' appearance following infection by *H. fraxineus* than susceptible families and in fact the difference between the two categories was nearly five-fold. Reasons for the small decline (3%) in stem form rating exhibited by some few tolerant families between years however, was not particularly unclear, but could have arisen from differences in how the resprouting was classified (if sprouting occurred higher in the tree following branch death vs. from the base of the tree); two different persons performed the assessments in 2015 and 2016.

It was evident also that large variation occurred between plant types (Ekebo and Uppsala), whereby plant stock that was originally grown in Ekebo held the highest mean stem form rating in both 2015

and 2016. Before establishing the trial, all seedlings were subjected to similar growing conditions; the main difference being that they had a difference of 1 year in age: Ekebo plants were 2-years-old and Uppsala plants were 1-year-old when the trial was established. Since it was found that the average number of stems for plant type Ekebo was higher than for Uppsala, it is possible that the additional year of growth have resulted in more stored energy than the younger plants, making it possible to sprout more new stems when infected by the disease. Significant differences were also found for three of the ten blocks in the experimental design. Reasons for this are unclear and edge effects unlikely as the trial itself was buffered by ash trees of a known susceptible genotype also originating from Ekebo.

Bud burst:
During both years, the variance for bud burst was largest within families, indicating that timing of bud burst does not depend on its genetics nor its susceptibility status, at least in this small population. In 2016, the bud burst assessment was conducted later in May which explains the higher ratings in all families compared to the previous year as many seedlings already had fully burst at the time of assessment. The large within-family variance may be attributable to the open-pollination of trees in the seed orchard, with only the identity of the mother clone known. Previous studies have found that the time of bud burst varies between susceptible and tolerant seedlings or clones; those with late flushing and late leaf shed being more susceptible than those with early flushing and earlier leaf shed (McKinney et al. 2011, Nielsen et al. 2017). However, in this study it is not possible to show any relationship between susceptibility to ash dieback and early leaf phenology (flushing).

The only significant difference found in the bud burst assessment were among blocks in 2016, where the ratings were slightly lower for blocks in one corner of the trial, block 9 and 10. The location might have affected the light availability, since the trial is surrounded with larger planted trees which could have shaded seedlings in those blocks.

Snogeholm

Overall, the clones established at the Snogeholm trial showed high survival. The small proportion (1.5%) of plants that died was not caused by *H. fraxineus*, but rather due to environmental factors that may have affected the establishment e.g. poor soil scarification and vegetative competition. Being this early in the trial, there is no clear differences yet between susceptible and tolerant clones of *F. excelsior,* nor how those clones will perform against the Asian *Fraxinus* species (*F. mandschurica, F. platypoda, F. spaethiana* and *F. japonica var.stenocarpa).* All clones, even the Asian *Fraxinus* species, showed symptoms of leaf necrosis in September, though four *F. excelsior* clones (3001, 3016, 3019, 3024) had all their ramets showing symptoms of the disease at the September assessment. The proportion of affected Asian seedlings underwent a drastic change throughout the season. The higher proportion of infected Asian seedlings in September reflects the increased proportion of infected seedlings overall in September, for all clones included in the trial. Therefore, it seems like the Asian clones, so far, preform as good as the *F. excelsior* clones. A recent study suggests that *H. fraxineus* is pathogenic to *F. mandschurica* but in that study it was only clarified that it can cause necrosis of *F. mandschurica* leaves and may not be the reason behind shoot dieback (Drenkhan et al. 2016). Despite many of the Asian seedlings exhibiting symptoms in this trial, they had the lowest HCR. Though leaves can be infected by *H. fraxineus,* it remains unclear if the pathogen will cause progressive and chronic damage to the stems as it does with *F. excelsior*. In

other work, *H. fraxineus* has been found to be a benign associate to the native Asian *Fraxinus* species behaving initially as an endophyte and switching to a saprotrophic phase during leaf senescence (Cleary et al. 2016). Whether *H. fraxineus* is pathogenic or not to the Asian *Fraxinus* species included in this trial will be discerned through follow-up assessments like that performed under this study. Though it is too early to draw any conclusions from the first assessments at Snogeholm, it provides important baseline information by which the early performance of *F. excelsior* clones selected for their putative resistance in wild-type populations can be assessed for their resistance against *H. fraxineus* and will serve as an important first step in further screening of genotypes that can be used for commercial propagation and future establishment of seed orchards.

Conclusions

In this thesis, the temporal dynamics of *F. excelsior* genotypes were evaluated during the first years of establishment on different scales: as progenies from known susceptible or tolerant mother clones, and as grafted clones from wild-type selections. In general, the work revealed a considerable increase in disease incidence and severity through the growing seasons and between years. Both selected tolerant and susceptible genotypes were largely affected by the disease. In general, progenies from known tolerant families performed better than progenies from susceptible families, suggesting that selecting for tolerant individuals will on average result in better performance. However, material initially considered tolerant also exhibited high susceptibility and damage which underscores the importance for continued monitoring of these genotypes in the long-term. However, around 50% of the progenies from tolerant families are so far showing superior performance and thereby are promising candidates for future selection to breeding or for establishment in seed orchards for commercial production of seed. Continued monitoring of the Alnarp trial and the current and future test populations established at Snogeholm will be important in order to screen individuals for their inherent resistance to the disease and support the development of a more resistant ash population for planting in Sweden.

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Appendix I

The codes found below are those that have been used to statistically test all mentioned variables (bud burst, health class rating and stem form). The names seen below is used as part of an example for how the calculations were done for one of the health class ratings. The example of the codes used in R is based on data which can be found in (Table 6) for better understanding of what has been conducted with the program.

When calculating the variance for the different factors, it was found that the interaction effects was not significant and the model became better (lower AIC) when the last part of the statistical design was excluded (i.e. interaction effects). The model used was therefore given as follows:

result0715.additiv <- lme(fixed=HCR0715~block+planttype,random=~1|family)

Before this step, using lme code, all NA values were removed from the dataset and categories such as block, family and plant type were converted into factors making it possible to use them in the calculations.

The code "summary" in R was used to show the variance for random effects, such as family (among family effects) and residuals (within family effects) in the trial:

summary(result0715.additiv)

The command "anova" showed the significance of fixed effects in the trial, such as block and plant type:

anova(result0715.additiv)

Table 7. Data, for health class rating at Alnarp trial, used when performed statistical calculations in R for health class rating

block	famno	planttype	family	HCR0715	HCR0716	HCR0916
1	101	1	1	2	2	2
2	101	1	1	1	3	3
2	101	1	1	3	3	3
3	101	1	1	3	3	3
4	101	1	1	1	3	3
4	101	1	1	2	3	3
5	101	1	1	3	4	4
6	101	1	1	3	5	5
6	101	1	1	1	1	2
7	101	1	1	3	3	3
8	101	1	1	2	2	3
8	101	1	1	3	3	3
9	101	1	1	5	5	5
10	101	1	1	3	5	5
1	104	1	4	2	2	2
2	104	1	4	1	1	2
3	104	1	4	5	5	5
4	104	1	4	1	2	2
5	104	1	4	3	3	3

5	104	1	4	2	1	2
6	104	1	4	2	1	2
6	104	1	4	2	2	2
7	104	1	4	1	2	2
7	104	1	4	2	1	2
8	104	1	4	2	2	2
8	104	1	4	5	5	5
9	104	1	4	5	5	5
10	104	1	4	2	2	2
10	104	1	4	3	4	4
1	106	1	6	3	3	3
1	106	1	6	3	3	4
2	106	1	6	2	3	4
2	106	1	6	3	4	4
3	106	1	6	3	3	3
3	106	1	6	3	4	4
4	106	1	6	3	4	4
4	106	1	6	3	3	4
5	106	1	6	3	4	4
5	106	1	6	3	3	3
6	106	1	6	2	2	3
6	106	1	6	3	4	5
7	106	1	6	5	5	5
7	106	1	6	5	5	5
8	106	1	6	3	4	4
8	106	1	6	1	2	2
9	106	1	6	3	3	4
9	106	1	6	5	5	5
10	106	1	6	3	2	3
10	106	1	6	3	2	3
10	106	1	6	3	2	3
1	114	1	14	3	1	3
2	114	1	14	2	1	2
2	114	1	14	1	2	2
3	114	1	14	1	1	2
3	114	1	14	2	1	2
4	114	1	14	2	2	2
4	114	1	14	3	3	3
5	114	1	14	3	3	4
6	114	1	14	2	2	2
7	114	1	14	1	2	2
8	114	1	14	1	2	3
8	114	1	14	NA	2	2
9	114	1	14	2	2	2
9	114	1	14	2	1	2
10	114	1	14	2	1	2
1	115	1	15	3	1	2
2	115	1	15	1	1	2

3	115	1	15	3	2	3
3	115	1	15	2	1	2
4	115	1	15	1	2	2
4	115	1	15	2	1	2
5	115	1	15	2	1	2
6	115	1	15	5	5	5
6	115	1	15	1	1	2
7	115	1	15	2	2	3
8	115	1	15	3	1	2
8	115	1	15	5	5	5
9	115	1	15	1	1	2
9	115	1	15	1	1	2
10	115	1	15	2	2	2
1	118	1	18	1	2	2
1	118	1	18	2	3	3
2	118	1	18	2	1	2
3	118	1	18	3	2	3
3	118	1	18	2	2	2
4	118	1	18	3	3	3
5	118	1	18	1	2	2
5	118	1	18	1	2	2
6	118	1	18	1	2	2
6	118	1	18	2	1	2
7	118	1	18	2	2	2
7	118	1	18	1	1	2
8	118	1	18	2	2	2
8	118	1	18	2	1	2
9	118	1	18	2	2	2
9	118	1	18	2	1	2
10	118	1	18	3	3	3
10	118	1	18	1	2	2
1	121	1	21	1	3	2
2	121	1	21	2	1	2
2	121	1	21	2	2	2
3	121	1	21	1	1	2
3	121	1	21	3	5	5
4	121	1	21	3	2	3
4	121	1	21	2	1	2
5	121	1	21	2	2	2
5	121	1	21	1	2	2
6	121	1	21	3	2	3
7	121	1	21	1	1	2
7	121	1	21	2	3	3
8	121	1	21	1	1	2
8	121	1	21	1	1	2
9	121	1	21	1	1	2
9	121	1	21	2	3	3
10	121	1	21	2	1	2

10	121	1	21	2	1	2
1	122	1	22	5	5	5
1	122	1	22	5	5	5
2	122	1	22	3	3	3
3	122	1	22	3	2	3
3	122	1	22	5	5	5
4	122	1	22	2	1	2
5	122	1	22	5	5	5
5	122	1	22	1	2	2
6	122	1	22	3	1	2
7	122	1	22	2	2	2
7	122	1	22	2	1	2
8	122	1	22	3	3	4
8	122	1	22	5	5	5
9	122	1	22	1	1	2
9	122	1	22	3	2	3
10	122	1	22	5	5	5
10	122	1	22	1	1	2
1	124	1	24	2	1	2
2	124	1	24	1	2	2
3	124	1	24	1	1	2
4	124	1	24	1	1	2
5	124	1	24	5	5	5
6	124	1	24	2	2	2
7	124	1	24	2	1	2
1	125	1	25	3	2	3
1	125	1	25	3	3	3
2	125	1	25	1	2	2
2	125	1	25	3	3	3
3	125	1	25	1	1	2
4	125	1	25	5	5	5
4	125	1	25	1	2	2
5	125	1	25	1	1	2
5	125	1	25	3	3	3
6	125	1	25	5	5	5
6	125	1	25	3	2	3
7	125	1	25	3	2	3
8	125	1	25	3	2	3
8	125	1	25	2	4	2
9	125	1	25	3	4	4
9	125	1	25	3	2	3
10	125	1	25	3	2	2
10	125	1	25	3	3	4
1	128	1	28	5	1	2
1	128	1	28	3	2	2
2	128	1	28	1	2	2
3	128	1	28	3	2	3
4	128	1	28	3	2	2

4	128	1	28	3	2	2
5	128	1	28	3	5	5
5	128	1	28	1	2	2
6	128	1	28	5	5	5
7	128	1	28	1	1	2
8	128	1	28	3	3	3
9	128	1	28	3	2	3
9	128	1	28	5	5	5
10	128	1	28	1	1	2
10	128	1	28	2	2	2
1	131	1	31	2	1	2
1	131	1	31	NA	NA	NA
2	131	1	31	1	3	3
2	131	1	31	5	5	5
3	131	1	31	1	3	3
3	131	1	31	1	1	2
4	131	1	31	2	1	2
4	131	1	31	2	1	3
5	131	1	31	5	5	5
5	131	1	31	2	1	2
6	131	1	31	2	1	2
6	131	1	31	3	2	3
7	131	1	31	1	2	3
8	131	1	31	2	1	2
9	131	1	31	2	1	1
9	131	1	31	2	3	4
10	131	1	31	5	5	5
1	201	2	1	1	5	5
2	201	2	1	3	2	2
3	201	2	1	2	1	2
4	201	2	1	3	3	2
5	201	2	1	5	5	5
6	201	2	1	3	2	3
7	201	2	1	3	2	2
8	201	2	1	3	3	3
9	201	2	1	2	2	2
10	201	2	1	2	1	2
1	204	2	4	2	2	2
2	204	2	4	1	1	2
3	204	2	4	2	2	2
4	204	2	4	1	1	2
5	204	2	4	1	1	2
6	204	2	4	2	2	3
7	204	2	4	2	2	2
8	204	2	4	1	2	2
9	204	2	4	3	5	5
10	204	2	4	1	1	1
1	206	2	6	2	1	2

2	206	2	6	1	1	2
2	206	2	6	3	2	3
3	206	2	6	2	2	2
4	206	2	6	3	3	3
5	206	2	6	1	2	3
6	206	2	6	5	5	5
6	206	2	6	3	2	3
7	206	2	6	3	2	3
8	206	2	6	5	5	5
9	206	2	6	5	5	5
10	206	2	6	3	3	4
1	212	2	12	5	5	5
3	212	2	12	3	2	3
5	212	2	12	3	3	3
8	212	2	12	4	3	3
10	212	2	12	1	1	2
1	214	2	14	2	2	2
2	214	2	14	2	1	2
3	214	2	14	3	2	2
4	214	2	14	1	1	2
4	214	2	14	2	2	2
5	214	2	14	2	2	2
6	214	2	14	2	1	2
7	214	2	14	2	2	2
7	214	2	14	1	2	2
8	214	2	14	1	2	2
9	214	2	14	1	2	2
9	214	2	14	2	2	2
10	214	2	14	1	1	2
10	214	2	14	2	2	2
1	215	2	15	1	1	2
2	215	2	15	3	1	2
3	215	2	15	1	2	3
4	215	2	15	1	2	2
5	215	2	15	1	2	2
6	215	2	15	1	1	2
7	215	2	15	2	1	2
7	215	2	15	1	1	2
8	215	2	15	2	2	2
9	215	2	15	1	1	2
10	215	2	15	5	5	5
1	218	2	18	2	2	2
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2	218	2	18	1	1	2
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3	218	2	18	2	2	2
4	218	2	18	1	2	2
5	218	2	18	1	2	2

6	218	2	18	1	1	2
6	218	2	18	2	1	2
7	218	2	18	1	1	2
7	218	2	18	1	1	2
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9	218	2	18	1	2	3
10	218	2	18	3	1	2
1	221	2	21	1	2	2
1	221	2	21	4	3	4
2	221	2	21	3	2	3
2	221	2	21	1	1	2
3	221	2	21	2	1	2
3	221	2	21	3	3	2
4	221	2	21	3	2	3
4	221	2	21	1	2	2
5	221	2	21	1	1	2
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6	221	2	21	3	2	3
6	221	2	21	1	1	2
7	221	2	21	2	2	3
7	221	2	21	2	1	2
8	221	2	21	1	1	2
8	221	2	21	2	2	3
9	221	2	21	3	3	3
9	221	2	21	1	2	2
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10	221	2	21	2	1	2
1	222	2	22	3	3	3
2	222	2	22	1	2	2
3	222	2	22	3	3	3
7	222	2	22	2	2	2
1	225	2	25	1	2	2
2	225	2	25	1	3	3
3	225	2	25	1	2	2
4	225	2	25	1	1	2
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6	225	2	25	1	2	2
7	225	2	25	2	1	2
8	225	2	25	2	3	4
9	225	2	25	5	5	5
10	225	2	25	3	5	5