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– current status and future prospects for Fraxinus excelsior

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Abstract

Fraxinus excelsior has great ecological, cultural and a somewhat economical importance in Sweden despite its low abundance. The future of common ash is however seriously threatened by the ascomycete fungus *Hymenoscyphus fraxineus* rapidly spreading across Europe and currently affecting ash throughout Sweden. To evaluate this affect inventories of ash stands were conducted during the summer of 2014 in the counties of Jönköping and Östergötland. Plot inventory (which incorporated all species in plots) was performed in stands of forest setting while in a second inventory that included avenues, pasture land and one seed stand, all ash were inventoried. The inventories contained assessments of health (i.e. visible crown damage), height and crown class and measurements of diameter at breast height. The results showed that the majority of ash in this area were highly susceptible to ash dieback and will probably die within a few years time; the seriously affected ash with more than 65 % visible crown dieback was 82 % in the plot inventory (60 % dead/almost dead) and 64 % in the avenues, pasture land and seed stand (23 % dead/almost dead). The results also showed that a small proportion of ash were seemingly more resistant ash (crown dieback less than 35 %); only 2 % (2.6 % if healthier ash found outside plots were included) in the entire plot inventory and 12 % in the avenues, pasture land and seed stand. The small and scattered distribution of these more resistant ash trees strongly points to a future probable risk of genetic diversity loss due to genetic drift and inbreeding. Relationships among different size classes and higher or lower susceptibility were explored in the data from the plot inventory (random sample) but no clear trends were found. A significantly higher proportion of healthier *Ulmus glabra* trees were found compared to common ash in the surveyed sites. This is inconsistent with the fact that elm is ranked as being more threatened to Dutch elm disease compared to ash to ash decline on the Red list of endangered species. The results from the sites herein were instead indicating a vast deterioration of the ash population and that, if these results are representative for the situation in Sweden, the current ranking of ash on the Red list should be revised. An additional aim of this survey was to locate more resistant ash trees that could be used as potential candidates for breeding. Of the 69 relatively resistant ash trees found in this survey 25 have been selected for scion collection and will, with others, be tested further. The summarized results from these inventories strongly points to the necessity of a breeding program to secure the future of ash and preserve enough genotypic variation for this tree species to cope with this aggressive invasive pathogen, as well as future challenges.

Keywords: Fraxinus excelsior, common ash, ash dieback, *Hymenoscyphus fraxineus*, *Ulmus glabra*

Table of contents

Abbreviations

Dbh	Diameter at breast height
H0-5	Health class 0-5
Ibid.	Same source as previously stated

1 Introduction

Ash dieback, caused by the ascomycete *Hymenoscyphus fraxineus*, is currently threatening the existence of *Fraxinus excelsior* in Sweden as well as in other European countries. This introduction will first focus on common ash, occurrence, dispersal and the importance of ash to humans and ecosystems. The second focus will be on the virulent fungus *H. fraxineus* and the spread of this pathogen. This takes us to the third focal point; the presence of more resistant ash and the potential of both a breeding program based on such tolerant individuals as well as other promising facilities. The central aim of this paper is to illustrate the current situation for common ash in two Swedish counties to examine the impact of ash dieback at a landscape level and to evaluate patterns at different levels of health. This will provide a tool for comparing the ash situation to other species and countries and analyse future prospects of ash. The additional aim is to locate common ash more resistant to *H. fraxineus* in diseased stands for the possible use in a future breeding program.

1.1 *Fraxinus excelsior*

Common ash (hereafter referred to simply as ash) found its way into Sweden from the south approximately 9000 years ago (Almgren *et al.*, 2003) and now generally occurs in Götaland and Svealand (Löf *et al.*, 2008) up to the northern border of "Limes Norrlandicus". Pure stands of ash are rare but do exist, instead ash is more commonly found in avenues, pasture land, as farm trees and in mixed forests with other tree species (Skogsstyrelsen, 2013). The distribution of ash in Sweden is mostly fragmented and sparse, the species only comprises 0.6 % of the total standing volume of all species in Götaland and even less in Svealand (0.1 %) (Skogsstyrelsen, 2014). The total estimated standing volume for ash in Sweden is 6.1 million m^3sk (ibid.). In the counties of Östergötland and Jönköping, the focus of this thesis, there is a low frequency of ash, about 0.4 % and 0.1 % of the standing volume consists of ash in each county, respectively (Skogsdata, 2014).

Ash seedlings and saplings are shade tolerant but become increasingly light demanding with increased age (Dobrowolska *et al.*, 2011) and hence succumb to critical light conditions (Emborg, 1997). The scattered distribution of this species is probably due to its specific demands to soil nutrients, water and climate (Dobrowolska *et al.*, 2011; Almgren *et al.*, 2003). Ash pollen as well as ash seeds are dispersed by wind (Dobrowolska *et al.*, 2011; Pliûra & Heuertz, 2003). Pollen and seed dispersal defines the gene flow pattern and determines, with other ecological factors, the future genetic structure of plant species (Loveless & Hamrick, 1984). Other cooperating factors for efficient gene flow in wind pollinated trees are synchronized flowering, population size, species mixture, density of canopy, wind conditions and distance to fellow species (Bochenek, 2011). The pollen movement in ash stands in Sweden and Britain was examined by Fraxigen (2005) where the average distance between mates was 41-72 m depending on the number of trees flowering. An investigation of more fragmented ash in Scotland had the mean distance of 328 m between mates (Bacles *et al.*, 2005). Ash seeds can be dispersed by wind more than 100 meters in the right circumstances (Wagner, 1997) and probably several kilometres in moving water (in stream simulation, 50 % of seeds dispersed >44 km) (Schmiedel & Tackenberg, 2013). The amount of seeds dispersed depend upon factors as dbh, gender (Dobrowolska *et al.*, 2011), years since previous mast year and various abiotic conditions (Fraxigen, 2005).

Genetic diversity can be measured by allelic richness or heterozygosity and reveals the variation of the genes within a population. Measurements of both were carried out by Fraxigen (2005) and they found that isolated ash populations had the lowest allelic richness and lower levels of heterozygosity compared to continuous populations. The isolation could be either due to higher latitude, bordering the northern range of ash, difference in time of flowering (higher altitude) or distance to neighbouring populations (ibid.). The authors also conclude that when ash is disintegrated in the landscape and the exchange of genes between populations become limited, the genetic differentiation is increased and these populations are more severely affected by genetic drift (ibid.). Genetic drift could lead to a diminished ability of a species to cope with future challenges (Hilfiker *et al.*, 2004). A lot of scientific research show evidence of genetic drift and inbreeding due to isolation (Li *et al.*, 2012; Fady *et al.*, 2008; Hirayama *et al.*, 2007; Hilfiker *et al.*, 2004) and there is also a theory of isolation by distance, meaning that kinship decreases between individuals with the logarithm of the distance (Born *et al.*, 2008). Compared to plants with short life cycles, trees are especially dependent on a broad genetic base to cope with different pathogens and climatic changes since their adaptation process takes a much longer time (McKinney *et al.*, 2014).

1.2 Values of preserving ash

1.2.1 Historical values

Ash has historically been a symbol of strength and virility in Europe (Bell et al, 2008) and in the Nordic mythology, Yggdrasil is the ash representing the foundation of the world. Throughout Europe, ash has historically and in some cases still, have a wide variety of uses. Bell et al. (2008) refers to the following examples;

- Fodder for animals during drought and winter from pollard trees - Ash foliage is to this day acknowledged as nutritious forage, especially used as food for nursing females, since it contains a lot of calcium and other micronutrients.
- Firewood - ”a king deserves an ash fire” (Bell *et al.*, 2008:10). The wood is considered an excellent firewood, with almost no smoke, high thermal value and shorter drying period than oak and beech.
- Medicinal - as a diuretic, laxative, styptics (bark), against fever, pain, impotence, rheumatism, gout and snakebite.
- Tools and arms – Such items were often made by ash wood, since it is very durable, hard and at the same time flexible. Tool handles can still be found made by ash wood.
- Fencing and rural equipment – These were often made of ash, due to its tough qualities and its ease to work with.
- Food and beverage

A lot of superstition has involved ash historically, often connected with healing or protection of children, finding love or fortune and with protection from snakes. Ash phenology has been used to predict the weather, sometimes in relation to oak. (Bell *et al.*, 2008)

1.2.2 Environmental values

Ash is a valuable tree growing in large parts of Europe (Pautasso *et al.*, 2013) and the species influence the forest both as a pioneer and a secondary species (Dobrowolska *et al.*, 2011). Compared to other tree species, ash litter has a lot of positive effects on the soil; high Ca and Mg concentrations, and high levels of N, P, K and S still available, making this litter one of the fastest decomposing (nutrient cycling) (Mitchell *et al.*, 2014). Ash is also distinguished by a thinner canopy, thus letting light come down to the understorey, enabling more species to grow there (Mitchell *et al.*, 2014; Emborg, 1997).

There are a lot of species depending on ash, but the scientific record on this is sparse (Pautasso *et al.*, 2013). Michell et al. (2014) reviewed European literature (both published and unpublished) and found 953 species linked to common ash. Out of these, there were 44 species obligate to ash (11 fungi, 29 invertebrates and

4 lichens) and 62 species were considered ”highly associated”, meaning the species hardly ever uses other tree species . When Mitchell et al (2014) analysed these results, with perspective to the species ash-association and protection rating, they found 69 species with the highest risk (code RED) and 169 species with high risk (code AMBER) of extinction/decline due to ash dieback. The species of code RED, 13 fungi, 6 bryophytes, 37 invertebrates and 13 lichens were recommended to receive the most attention and to attend for precautionary methods to avoid extinction.

In Sweden there are several threatened species with varied association to ash, for example the moss *Orthotrichum pallens*, the lichen *Pyrenula nitidella* and the fungus *Auricularia mesenterica* (Pihlgren *et al.*, 2010). In total, there are 180 known red listed species associated to ash and since ash bark has different qualities in pH and nutrition than the expected substitute, there are concerns that ash deficit will influence even more species (Skogsstyrelsen, 2013). A survey in Sweden found ash to host in average 0.7 red-listed lichen per tree and only *Ulmus glabra minor* exceeded ash in this (Thor *et al.*, 2010). This survey also found a positive weak correlation between abundance of lichens and diameter on ash (ibid.) An analysis of the epiphytic moss *Neckera pennata* by Roberge *et al.* (2011) concluded that the development of ash dieback increases the risk of this vulnerable species dying out. In contrast to studies emphasizing negative environmental consequences of ash dieback Heilmann-Clausen *et al.* (2013) point to positive effects of dead and dying ash in the landscape and diminish the threat of ash dieback on biodiversity.

1.2.3 Economical values

There are three native species of ash in Europe; *F. excelsior*, *F. ornus* and *F. augustifolia* (Fraxigen, 2005). In the economic perspective, common ash is the most demanded of these *Fraxinus* species (Pliûra & Heuertz, 2003). The wood is today used for sports equipment, furniture, flooring and veneer (Bell *et al.*, 2008; Pliûra & Heuertz, 2003). For some purposes, ash wood is exclusively required, for instance to manufacture Hurley sticks (Bell *et al.*, 2008).

1.3 Ash decline in Europe

Damaged ash stands were first observed in Poland around 1992 (Kowalski & Łukomska, 2005) and the disease has since spread to major parts of Europe including Lithuania, Denmark, Norway, Austria, Germany, France, Belgium and Great Britain (Pautasso *et al.*, 2013). In Lithuania the first outbreak of the disease was seen in 1995-1996 and has rapidly spread to presently affect all ash stands in the country and has reduced the area of ash stands by at least 40 % (Lygis *et al.*, 2014). Data from the Lithuanian State Forest Service show that 49 % of the ash trees died in the state forests during the period 2009-2013, representing almost 10 % per year decline (Lygis, personal communication)[1]. In addition to the amount of dying ash, the regeneration of ash is also severely affected in Lithuania (Lygis *et al.*, 2014). In clear cut stands previously dominated by ash this survey now found merely 0 – 21 % ash in the new emerging tree population and this was suggested to be caused by both poor vitality in seed trees as well as poor growth and vitality in the regeneration of ash induced by the ongoing disease (ibid.). A lack of management in these surveyed sites might also have contributed to the poor ash regeneration (Lygis *et al.*, 2014).

1.4 Ash decline in Sweden

The disease was first reported in Sweden in 2001 (Johansson *et al.*, 2009) and has since then spread very quickly. In two years ash dieback could be observed over the whole distribution area in Sweden (Bengtsson, 2014). An inventory in Götaland 2009 and 2010 of ash on forestland found that approximately 30 % of the ash trees (>10 cm dbh, n= 539) were either dead or severely damaged (Wulff & Hansson, 2011). In a survey of ancient ash in Västra Götaland trees affected by ash decline increased to 84 % in 2013 from 62 % and 77 % in 2009 and 2011 respectively (Bengtsson, 2014). In the different years of inventory, some trees completely healthy in previous survey, had died two years later and there were also some examples of trees appearing to recover, assumed to be related to excessive production of adventitious shoots (Bengtsson, 2014; Bengtsson *et al.*, 2013). In 2010, *Fraxinus excelsior* together with *Ulmus glabra* was added to the Red list of endangered species in Sweden as vulnerable (VU) due to the progress of ash decline and Dutch elm disease respectively (Pihlgren *et al.*, 2010).

1.5 Hymenoscyphus fraxineus

Hymenoscyphus fraxineus was previously named dually, the asexual morph *Chalara fraxinea*, with the sexual form *Hymenoscyphus pseudoalbidus* (Baral *et al.*, 2014) and this ascomycete is confirmed to be the causal agent of ash decline (Queloz *et al.*, 2011; Kowalski, 2006). The tree-level symptoms of ash decline are infections in cambium and brown-orange necrosis and cankers emerging to the bark (Schumacher *et al.*, 2010), wilting of shoots and branches and since the trees often respond with development of epicormic shoots, the sick trees prevalently gets a tufted appearance (McKinney *et al.*, 2014). Skovsgaard *et al.* (2010) found a

1. Vaidotas Lygis, PhD, Institute of Botany of Nature Research Centre, 2014-12-04

strong correlation between dieback and the occurrence of cankers in the crown and concluded that this could be the primary symptom since wilting was not seen on all trees with dieback. Otherwise symptoms on the leaves are associated with the first obvious signs of the pathogen with lesions on veins and petioles, usually with subsequent wilting (Kräutler & Kirisits, 2012). Collar lesions on diseased ash have been attributed to both *Hymenoscyphus fraxineus* and *Armillaria spp*, but *Armillaria* is probably the secondary pathogen on a host impaired by the primary pathogen *H. fraxineus* (Husson *et al.*, 2012; Bakys *et al.*, 2011; Skovsgaard *et al.*, 2010).

1.5.1 Dispersal

H. fraxineus infect the trees with ascospores (Cleary *et al.*, 2013; Gross *et al.*, 2013; Timmermann *et al.*, 2011) that are dispersed in the summer from apothecia formed on litter from earlier infected leaves (Gross *et al.*, 2012). The ascospores are windborne and the highest amount of dispersal occur in the end of July to mid-august when values between 6 and 8 in the morning has been recorded with the maximum amount of 4.7 million spores per cubic metre (Timmermann *et al.*, 2011). The dispersal is calculated to be on average 75 km per year in the central Europe (Gross *et al.*, 2013) and in Norway it was reported to move from east to west approximately 30 km in one year (Timmermann *et al.*, 2011).

1.5.2 Entering and spreading in the host

The ascospores released in the summer reach new hosts, attach to leaves and the pathogen enter by means of appressoria formed from these germinating ascospores (Cleary *et al.*, 2013; Gross *et al.*, 2012). The fungus spreads from the infection point through petioles into the stem, this is most often preceded by wilting of the distal leaves (Cleary *et al.*, 2013; Gross *et al.*, 2013). In the stem *H. fraxineus* spread in all parts and directions of the wood tissues (Schumacher *et al.*, 2010). Growth is most efficient along the pith and vessels (vertically) and also in the ray parenchyma (horizontally) and eventually, with a slower process along the radial rays, the pathogen reach the cambium and phloem, showing the external discoloration in the outer bark (Schumacher *et al.*, 2010). The pathogen may also be able to enter directly through the lenticels or wounds in the bark (Kräutler *et al.*, 2015; Husson *et al.*, 2012). The fungus have also been detected in root tissue, but only on stem infected ash (Schumacher *et al.*, 2010).

1.5.3 Mitoviruses in *H. fraxineus*

Virus in plant pathogenic fungi can cause hypo virulence, making the pathogen less virulent to the host. Schoebel *et al.* (2014) examined and found the same strain of mitovirus (virus located in mitochondria of the fungi) in 90 % of *H. fraxineus* isolates from two different diseased ash populations in Switzerland and in one isolate from Japan. In this study, the strains of virus differed greatly genetically in the isolates from other countries. Since the mitovirus was so frequent, the authors assumed no hypo virulence existed yet, but results show the mitovirus genetic material alters rapidly so it could possibly evolve to reducing virulence in *H. fraxineus* in the future (ibid.) This kind of evolvement have been detected in other plant pathogenic fungi (Khalifa & Pearson, 2013; Wu *et al.*, 2007; Polashock & Hillman, 1994). Heiniger and Rigling (1994) described a virus inducing hypovirulence in *Cryphonectria parasitica* in some parts of Europe while Linder-Basso *et al.* (2005) examined the most common virus on *Cryphonectria parasitica* in North America, not especially affecting the virulence of the pathogen.

1.6 Characteristics of unhealthy vs. healthy ash

Ash decline affect all ash in the concerned areas (Schumacher *et al.*, 2010; Kirisits *et al.*, 2008; Kowalski, 2006) but some studies have seen a connection between various factors and the progression of ash dieback. In 15-year-old ash stands, trees without signs of dieback generally had larger DBH than the trees with signs of dieback on the main stem (Skovsgaard *et al.*, 2010). Since ash dieback largely affects the increment growth this difference in dbh could derive from the disease, but the authors also point out that less vigorous ash, due to other causes, would probably be more susceptible to ash decline (ibid.). In a survey of ancient ash in Västra Götaland, a significant relation between larger girth and better health was seen and no ash with girth less than 140 cm were healthy (Bengtsson, 2014). This study further examined the effect of shaded, semi open or open location on tree health but found no correlation (ibid.). Also lesions on the trunks were found to be more severe on ash of younger age probably due to their thinner bark (Husson *et al.*, 2012).

In addition to visually better health in larger trees the disease has been found to develop more slowly in larger trees compared to smaller (Gross *et al.*, 2013) and consequently also more slowly in older trees compared to younger (Keßler, 2012). Another relation found by Wulff and Hansson (2011) was a larger proportion of dead ash in stands with high proportion ash compared to stands with a low proportion ash in the species composition. In contrast, the proportion of dying ash (crown dieback between 60 % - 99 %) was greater in the stands with a low proportion of ash in the species composition (ibid.).

1.7 Resistance

Some ash trees in declining stands have been observed to cope better, with less severe symptoms and a much slower progression of lesions (Husson *et al.*, 2012; Keßler, 2012; Bakys *et al.*, 2009; Johansson *et al.*, 2009). Differences in disease expression between different provenances has also been reported (Enderle *et al.*, 2013; Lösing, 2013).This could be due to an active defence of trees producing chemical substances to fight pathogens (Eyles *et al.*, 2010; Johansson *et al.*, 2009). Several studies of clones and progenies have shown evidence of high heritability in the trait of more resistance and genetic variation in the susceptibility of ash, where some clones/progenies show signs of resistance with no significant effect of environment (Enderle *et al.*, 2014; Lobo *et al.*, 2014; Stener, 2013; Kirisits & Freinschlag, 2012; Kjaer *et al.*, 2012; McKinney *et al.*, 2011; Stener, 2007). In contrast, Pliura *et al.* (2011) detected a significant effect of localities on tree health in their progeny trial and suggested that the cause of this could be diverging growth conditions or infection pressure on the different sites. However, the synergy between genotype and habitat in this study was significant for tree health only at mid survey (after 4 years). After an additional four years this relationship was non-significant, indicating tree health in infected areas foremost being a genetic trait (ibid.).

Similar to other studies (Kjaer *et al.*, 2012; McKinney *et al.*, 2011; Pliura *et al.*, 2011), Stener (2013) did not find any clones to be completely resistant, instead the mean damage score (from 0 to 9, where 9 is most damaged) ranged from 1.5 to 5.5 between the different clones. Thus even the most resistant ash trees are affected by the pathogen but somehow manage to cope. The continuous distribution of resistance signifies the characteristics of quantitative traits, meaning several genes are involved forming this phenotype (McKinney *et al.*, 2014). Besides the limited level of resistance, the proportion of more resistant ash in studies is low (Lobo *et al.*, 2014; Kjaer *et al.*, 2012; McKinney *et al.*, 2011) for instance one out of 39 clones in the study by McKinney *et al.* (2011) had less than 10 % average crown damage.

Speculations on resistance versus disease escape have occurred. McKinney *et al.* (2011) postulated a relationship between shorter growing season and less susceptible trees and assumed this could be due to disease escape; earlier senescence in ash could avoid the pathogens peak infection pressure. This was further investigated by McKinney *et al.* (2012) through inoculation of infected plugs on the four most susceptible and four least susceptible clones from the previous survey (see above). Since the same pattern of disease development was detected, where the more resistant clones had a significantly lower necrosis advancement than the susceptible clones, it seems to be evident that resistance due to active tree defence against *H. fraxineus* exists (ibid.).

1.8 Actions against ash decline

Ash has a scattered distribution in most countries in Europe with sporadic occurrence of pure ash stands (Bell *et al.*, 2008). Ballian *et al.* (2008) describes a fragmented distribution of ash (without the presence of *H. fraxineus*) in the forests and landscapes of Bosnia and Hercegovina and that *F. excelsior* is being further diminished due to logging of this valuable tree. The examined populations in this study displayed a large amount of homozygotes and the probable cause was considered to be inbreeding (ibid.).

In Sweden ash is also fragmented in the forests and landscape and it is one of our least common species that form stands (Wulff & Hansson, 2011; Almgren *et al.*, 2003). Despite this, ash still has economic and environmental importance as a noble hardwood species (Stener, 2013). Small fragmented populations are known to be more vulnerable to the effects of genetic drift and to inbreeding (Ouborg *et al.*, 2006) which further disrupts the reproduction system (Frankham, 2003). The threat of extinction is hence more evident for solitary populations but a supplement of new genes can revolve this progression (Frankham, 2005). The potential of a breeding program to revive inbred populations is one aspect but results also show resistance against *H. fraxineus* to be heritable (Lobo *et al.*, 2014; Kjaer *et al.*, 2012; Pliura *et al.*, 2011), therefore it would be possible to breed on these qualities. Kjaer *et al.* (2012) argues in favour of a breeding program of resistant ash due to its low frequency and scattered distribution in the landscape. Pautasso *et al.* (2013) fears that the pathogen, due to its adapting ability and sexual reproduction, might in time defeat the resistant trees defence and hence argues for a genetic diverse breeding and large selection of candidates. Stener (2012) suggests a test of resistance, in which grafts from the healthy looking specimens should be inoculated with the fungus and the ones remaining most vigorous after this test could, if they are of sufficient number, be utilized as mother trees, thus providing a future ash stock. Also Kjaer *et al.* (2012) stressed the importance both of investigating the resistance potential of genotypes for a breeding program and of, for this purpose, locating a large sample of more resistant ash to provide for a broad genetic base.

In addition to the fragmented distribution and ash dieback diminishing the vitality and existence of ash, logging is contributing to the population decline in Europe. Extensive logging is occurring in order to harvest ash before the decline diminishes the timber value (Lobo *et al.*, 2014). The necessity to locate and thereafter collect samples of more resistant ash is therefore urgently needed.

Besides genetic control, other research on chemical and/or biological control for the disease are being pursued. Fungicides, more environmentally-friendly, exists that delay the progress of *H. fraxineus* (Dal Maso *et al.*, 2014) but are not an option today for large scale usage since such entail great expenditure and time (Dal

Maso *et al.*, 2014; Lösing, 2013). The general consensus is instead that breeding on the trait of more resistance is the best option to deal with this invasive pathogen (Lobo *et al.*, 2014; McKinney *et al.*, 2014; Pliura *et al.*, 2011). Work is in progress to understand the mechanisms with this trait and the quantitative genetics involved (McKinney *et al.*, 2012) and continued work in a breeding program would add further insight into this knowledge gap.

1.9 Objectives

The main purpose of this thesis is to evaluate the current situation and examine the future prospects of ash in Sweden. In order to achieve this, a picture of the present situation of the impact of ash dieback is needed. This will provide a foundation for evaluating the extent of damage to ash among different levels in the forest structure, for comparison with other species and further to speculate in the future for ash as a landscape tree species in Sweden.

Specific objectives:

1. What is the impact of ash dieback on ash stands in Jönköping and Östergötland Counties?
2. Are there any size factors associated with more resistant ash versus more susceptible ash?
3. What are the future prospects for ash in this area?

2 Materials and Methods

2.1 Selection of ash stands

The ash stands were mainly selected from data files obtained from the Swedish Forest Agency (key habitats and seed stands) and The County Administrative Board (avenues and nature reserves) in Östergötland and Jönköping counties. Information about all surveyed sites are displayed in Table 1 and Figure 1. The key habitats were chosen with the criteria of at least 50 % proportion of ash. The nature reserves and avenues with the largest number of ash were chosen and all of the seed stands in the county area. The two ash stands in the Ecoparc at Omberg was selected with the aid from Krister Samuelsson, Sveaskog, who had knowledge of the abundance of ash in this area. An Ecoparc is a larger area of land with nature conservational, recreational and economical aims, in that order.

Table 1. *Information about all sites surveyed in Östergötland (E) and in Jönköping (F) counties.*

Site No	Site name	County	Location (Sweref 99 TM)	Elevation (m)	Site category	Species composition (plots)[1]	Total no. of trees registered[2]	All trees in plots	Area (ha)
1	Västergården	E	N6495019-E504433	140	Key habitat	Fre(33 %)Cory(22 %) Sorb(15 %)Acpl(14 %)	222	222	0.7
2	V gröndalen	E	N6418410-E502679	220	Key habitat	Fre(55 %)Ulm(12 %) Sorb(8 %)Acpl(6 %)	243	243	0.8
3	Brohagen	E	N6460591-E543390	90	Pasture/Key habitat	Fre(54 %)Acpl(11 %) Cory(9 %)	97	96	2.7
4	Fäktahål	E	N6442050-E473662	140	Key habitat	Fre(47 %)Sorb(12 %) Ulm(10 %)Acpl(6 %)	283	283	1.7[3]
5	Strandsraviner	F	N6410224-E457381	250	Key habitat	Fre(82 %)Ulm(6 %) Sorb(5 %)Cory(2 %)	825	823	4.6[3]
6	Ingarp	F	N6411519-E466645	245	Key habitat	Fre(82 %)Qur(4 %) Pop(4 %)Sorb(4 %)	219	219	0.9
7	Sutarp	F	N6438966-E479042	240	Key habitat	Fre(50 %)Aln(41 %)	82	81	2.1
8	Ost Lundtorp	F	N6437920-E471823	140	Key habitat	Fre(50 %)Ulm(24 %) Cory(18 %)	185	185	1.6[3]
9	Dintestorp	F	N6430702-E429620	275	Key habitat	Fre(73 %)Aln(12 %) Acpl(7 %)Cory(6 %)	199	198	3.4[3]
10	Skämningsfors	F	N6440916-E452687	145	Avenue + forest	Fre(64 %)Acpl(15 %) Ulm(4 %) (in forest)	155	143	2.1[3]
11	Bondberget	F	N6404267-E455213	165	Nature reserve	Fre(68 %)Sorb(11 %) Bet(6 %)Cory(6 %) Qur(4 %)	255	227	6.8[3]
12	Ingaryd	F	N6397147-E458515	230	Nature reserve	Fre(62 %)Qur(23 %) Bet(15 %)	50	14	2.3[3]
13	Borsaskögle	F	N6347303-E515013	270	Nature reserve	Fre(63 %)Acpl(16 %) Ulm(13 %)Cory(4 %)	134	134	0.9[3]
14	Bollbynäs	F	N6353321-E402625	220	Seed stand (former)	Fre(67 %)Sorb(10 %) Cory(6 %)Acpl(3 %)	198	198	0.7[3]
15	Smala betet	F	N6432324-E461163	125	Seed stand (former)	Fre(92 %)A.alba(8 %)	30	26	1.0
16/ 17	Omberg	E	N6465620-E479934	200	Ecopark	Fre(30 %)Sambuc(17 %) Ulm(14 %)Prun(14 %) Acpl(8 %)	156 / 1547	153 / 1547	0.9[3]/ 4.6[3]
18	Pilalandet	F	N6432862-E461614	120	Seed stand	Fre (only ash surveyed)	85	0	2.2[3]
19	Lövhult	F	N6390270-E485948	295	Nature reserve	Fre (only ash surveyed)	20	0	2.2[3]
					Total	Fre(53 %)Ulm((%) Sorb(8 %)Acpl(6 %)	4985	4792	

1. Fre=Fraxinus excelsior; Ulm= Ulmus glabra spp; Acpl=Acer spp; Cory= Corylus avellana; Sorb=Sorbus spp; Prun=Prunus spp; Bet=Betula spp; Aln=Alnus spp; Qur=Quercus spp; Pop=Populus spp, Sambuc=Sambucus spp.

2. All trees surveyed on site, including trees of height class 1= < 1.3 m.

3. Area measured in ArcGIS Collector, other area information from Swedish Forest Agency (Skogsstyrelsen).

Figure 1. Location of the surveyed sites with inset map showing Östergötland and Jönköping Counties positions in Sweden.

2.2 Inventories

The inventories were made during June and July 2014 in the selected stands.

Two types of inventories were conducted depending on environmental circumstances (described further in the next pages);

1. Plot inventory
2. Inventory of all ash

For recording the data, the ArcGIS collector app was used. Each ash stand was assigned a unique ID number which was recorded at each location point along with the measurements and assessments described below in the plot inventory.

2.2.1 Plot inventory

For each of the stands, a grid of plots were constructed using a GPS, Garmin Oregon 650t. The transects started 15 m from the edge of the stand, with 25 meters between the plot centres, using the GPS both for correct direction in transects and for setting the direction 90º in relation to previous transect. The criteria for a plot to be measured included; at least seven trees within plot area, greater than 20 % ash in total species composition (including trees with dbh< 1.3 m) and located more than 15 m from the stand edge. Some areas were not surveyed due to their inaccessibility (ravines). The plot inventory was performed using 5.64 m radius (100 m²), recording all species within and making measurements and assessments according to Table 2.

Table 2. *All measurements and assessments made in the plot inventory.*

	Height class 1 (<1.3 m.)[1]	Height class 2-6 (1.3- >20 m.)[1]
Species	All species of trees and shrubs	All species of trees and shrubs
Dbh		Dbh measured to the nearest 1 cm.
Crown class		Assessed crown class[2]
Health class		Assessed visible crown dieback

1. Height classes from 1-6. 1= <1.3 m; 2= 1.3-5 m; 3=5-10 m; 4= 10-15 m; 5= 15-20 m; 6= >20 m.
2. Crown class – was assessed in the classes; dominant (in top of the canopy), codominant (in canopy), intermediate (below canopy) and suppressed (trees of restrained growth).

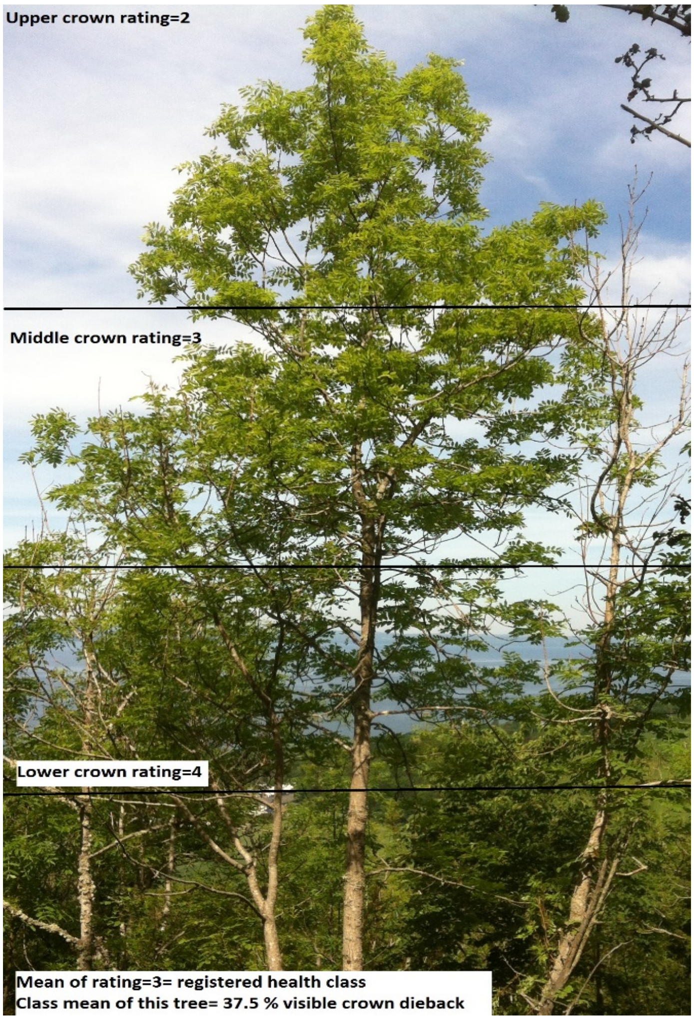

Figure 2. Health class assessment using rating system by Kirisits and Freinschlag (2012). (Photo: Sara Strandberg)

Health class – was assessed in a rating system, in most parts assigned to Kirisits and Freinschlag (2012), dividing the tree in three parts, rating each third in a scale from 0 to 6, 0 stand for no visible dieback on each third, 6 representing 100 % visible dieback on each third (see figure 2). 100 % visible crown dieback can either mean completely dead parts of the crown or nothing but epicormic shoots. The mean of the three ratings was calculated and registered as a health class in a scale from 0 to 5 according to Table 3. Examples of trees in different health classes are displayed in Figure 3.

Table 3. *Health class assessment in according to Kirisits and Freinschlag (2012), with some modification dividing the tree crown in three parts, assessing each third according to the first two columns. The mean of each third results in the final registered health class where health class 0 is the healthiest appearance and health class 5 is a crown with no or almost no living tissue left.*

Rate of each third	*Visible dieback of each third*	*Class mean*	*Mean of each third between*	*Registered health class*	*Mean rating based on class mean*
0	No dieback	0 %	0-0.9	0	0 % - 4.2 %
1	< 5 %	2.5 %	1-1.9	1	2.5 % - 12.5 %[1]
2	> 5 % to 20 %	12.5 %	2-2.9	2	12.5 % - 35 %
3	> 20 % to 50 %	35 %	3-3.9	3	35 % - 55 %
4	> 50 % to 80 %	65 %	4-4.9	4	65 % - 81.7 %
5	> 80 % to 100 %	90 %	5-6	5	80 % - 100 %
6	dead	100 %			

1. This mean rating is not correct in case one of the third ratings ≥4, for example: (0+0+4)/3=1.33-mean rating 21.6 %, was registered as health class 1 but should be registered as health class 2; (0+0+5)/3=1.66- mean rating 31.66 %, should be registered as health class 2.

Figure 3. Examples of ash trees in the different health classes 0-5. See table 2 for health class assessment. (Photo: Sara Strandberg)

2.2.2 Inventory of all ash - no random sampling

On sites that were too sparsely populated for plot inventories (i.e. one seed stand, avenues and pastureland), all ash trees on the site were recorded. In this inventory there was no random selection of the registered trees. This inventory is herein referred to as “inventory of all ash” to separate it from the plot inventories. The measurements and assessments in this inventory were equal to the plot inventory (see table 2) with the exception that it was made exclusively on ash.

2.2.3 Search for healthier ash

Since an additional goal of these inventories was to find relatively resistant ash genotypes, if such a tree, greater than 5 m in height, was found outside plots, it was recorded with the same measurements/ assessments as in the inventories. All ash in health class 0-2 (i.e. with less than 35 % visible crown dieback) greater than 5 meters in height within plots or outside, were photographed, marked with ribbons and tags and the coordinates of their location were registered with the gps. The recorded information of these healthier ash trees was sent on for further evaluation as candidates for scion collection.

2.2.4 Statistical analyses

Since the plots represents a random sample from the total population, i.e. the site, the analyses were exclusively made on the data from the plot inventory. Since all assessments/measurements were made only on trees taller than 1.3 m, the trees in height class 1 (i.e. <1.3 m) were excluded in all presentation of the results, except with respect to the species composition, where all counted species were included. All analyses were made on data within sites, since the conditions between sites were diverse in terms of soil, water and forest structure. Sometimes the total plot inventory data were analysed for comparison with the results from the individual sites. The data from the plot inventory within sites was not homogeneous and to be able to analyse the small data sets of ash in some groups, for example in health class 0-2, all the variables were grouped further. The constitution of the groups were defined after observations of frequency tables and adjusted to fit the data from the majority of the sites. All statistical analyses were conducted in Minitab®17.1.0.

A Chi square test for association was used for analysing differences in health class distribution in the top height classes and also for analysing differences between ash and elm (and within the group of elm) in the distribution of health and size classes. Despite the large clustering of groups there was still too little data in several sites for valid results. These sites were therefore excluded from the presentation of these results. Fisher's exact test was used for analysing differences in size distribution between health class 0-2 and health class 5 since the clusters of groups in the majority of the sites were too small for a valid chi-square test. To analyse relations between variables in the different sites of the plot inventory a Spearman's ranked correlation test was conducted. The ranked variables within sites in this test were; the mean values of health and size classes; the proportion of dead and almost dead ash of the total amount of assessed ash; the proportion of ash in the total species composition and also the total number of measured/assessed ash. Descriptive statistics were made on the data from the plot inventory and on the data from the inventory of all ash.

3 Results

3.1 Sites of more dense composition- plot inventory

Out of the 17 sites where plot inventory was possible, in five of them no healthier ash (health class 0-2) was found at all. In the other sites twice the amount was found in plots as outside plots. Bollbynäs (site no. 14) looks like a relatively healthy site compared to the other sites (see table 4), with the highest number of ash in health class 0-2 and a lower value of mean health class compared to the other sites. This site differed in forest structure from the other sites. In all the other sites, the small trees formed an understorey, with a shading canopy above. However, in this site there was no overstorey in most parts.

Table 4. *Information about all sites in plot inventory. Number of ash in the healthier classes (H0-2), within and outside plots and mean values from all ash in each site in plot inventory.*

Site No.	Site name	No. of plots	Measured/ assessed ash[1]	No. of ash in health class 0-2 in plots (outside plot)	Mean health class (SE mean)[1]	Mean height class (SE mean)[1]	Mean dbh (SE mean)[1]
1	Västergården	6	49	2 (0)	4.20 (0.13)	3.53 (0.23)	13.23 (2.34)
2	V gröndalen	4	66	0 (0)	4.73 (0.08)	2.91 (0.13)	4.64 (0.62)
3	Brohagen	6	12	0 (1)	4.09 (0.25)	4.82 (0.12)	52.61 (5.57)
4	Fäktahål	6	83	0 (0)	4.71 (0.06)	3.72 (0.15)	9.50 (0.91)
5	Strandsraviner	24	540	6 (2)	4.52 (0.03)	3.35 (0.05)	7.70 (0.29)
6	Ingarp	5	131	0 (0)	4.26 (0.06)	2.35 (0.06)	5.60 (1.11)
7	Sutarp	6	21	1 (1)	4.33 (0.20)	4.48 (0.30)	22.75 (3.75)
8	Ost Lundtorp	9	92	0 (0)	4.35 (0.08)	3.86 (0.15)	16.80 (1.46)
9	Dintestorp	11	118	3 (1)	4.03 (0.09)	2.75 (0.10)	6.86 (0.90)
10	Skämningsfors	9	75	2(0)	4.11 (0.10)	3.96 (0.19)	15.12 (1.54)
11	Bondberget	11	137	3 (4)	4.18 (0.08)	3.65 (0.10)	10.20 (1.02)
12	Ingaryd	1	8	0 (0)	3.5 (0.27)	2.63 (0.18)	4.42 (0.89)
13	Borsaskögle	5	47	2 (0)	4.02 (0.14)	3.09 (0.20)	8.10 (1.45)
14	Bollbynäs	8	49	7 (0)	3.43 (0.14)	2.78 (0.19)	9.08 (2.03)
15	Smala betet	2	10	1 (4)	3.4 (0.27)	5.7 (0.15)	28.30 (2.77)
16	Omberg 0	21	321	4 (0)	4.64 (0.04)	4.01 (0.07)	8.76 (0.27)
17	Omberg 1	5	76	1 (3)	4.72 (0.08)	3.79 (0.16)	10.89 (0.80)
	Total	139	1834	32 (16)	4.40 (0.02)	3.46 (0.03)	9.46 (0.25)

[1] Only ash with height > 1.3 m.

The seed trees in this former seed stand had been logged and hence only one of the seven healthier trees in site no. 14 is above 5 meters in height. The only other sites with a mean health value below 4.0 (sites no. 12 and 15) consisted of very small datasets (see measured/assessed ash in table 4) and the latter site was also a former seed stand which in most parts had been overgrown by *Abies alba*.

The sites in this plot inventory varied in forest structure and in distribution of height class and diameter at breast height, as seen in the mean values for those respective variables in Table 3. A majority of these sites were of dense forest structure, with a compact undergrowth, hence a low mean value of height and dbh. Exceptions to this were sites no. 3, 7 and 15, all having small sample sizes and also less dense forest structure than the other sites in this inventory (see table 4), nevertheless the number of trees were suffice to meet the criteria for surveying plot inventory.

3.1.1 Distribution of health class in plot inventory sites

Less than 2 % of the total number of ash in the plot inventory showed higher levels of natural resistance to the disease (health class 0-2). Within this group, 0.05 % were in the supreme health class 0 (1 ash out of 1834) and 0.27 % had visible crown dieback less than 12.5 % (health class 1, five ash trees). Even if the healthier ash found outside plots were included in the total, the frequency was still very low, only 2.6 % (table 5).

In the different sites with healthier ash within plots (11 sites), these health classes (H0-2) generally occurred less frequent, less than 5 %, with the exception of two sites, no. 14, earlier mentioned, with a different forest structure and no. 15, a site which also got a very large proportion of healthier ash if those outside plots were included. This site had of a very small sample size (n=10). Though if healthier ash outside plots were included, the frequency was still generally low. Apart from the sites with a dataset less than 21 ash trees, only site no. 14 had a proportion higher than 5 % (see table 4 and 5).

Health classes 4 and 5, representing ash trees with more than 65 % visible crown dieback (based on class mean, see table 3) were generally dominating the different sites, ranging from 64 to 98 % of the total number of assessed ash (see table 5). If the sites with small sample size, i.e. no. 12 and 15, were excluded, the only exception were the earlier mentioned site no. 14, Bollbynäs. In the plot inventory, 82 % (4 out of 5 ash trees) of the total number of ash surveyed were in health classes 4 and 5.

Health class 5 had the highest proportion of the ash population compared to the other health classes in 12 out of the 17 sites and the total frequency of this health class in the entire plot inventory was 60 %. This proportion of ash is hence dead or almost dead.

Comparing the frequencies of ash in suppressed/intermediate crown class and with dbh less than 5 cm in the different sites somewhat illustrates the forest structures of the sites (see table 5). Out of the total plot inventory 74 % were classified as suppressed/intermediate and 44 % were small trees (diameter at breast height < 5 cm).

Table 5. *Frequency of health class and frequency of ash in some size groups in the different sites in plot inventory. The empty space in health class 0-2 equals zero.*

		Frequency of ash in health class H (%)[1]					Frequency of ash in size groups (%)[1]	
Site No.	**Site name**	**H0-2**	**H0-2 including healthy ash outside plots**	**H3**	**H4**	**H5**	**Suppressed/ Intermedi-ate**	**Dbh<5 cm**
1	Västergården	4.1	4.1	22.5	12.8	51.0	78	44
2	V gröndalen			9.0	9.0	81.8	97	65
3	Brohagen		8.3	27.3	36.4	36.4	27	0
4	Fäktahål			2.4	24.4	73.2	77	37
5	Strandsraviner	1.1	1.5	10.7	23.5	64.6	79	44
6	Ingarp			16.8	40.5	42.7	98	81
7	Sutarp	4.8	9.1	14.3	23.8	57.1	38	24
8	Ost Lundtorp			16.3	32.6	51.1	61	30
9	Dintestorp	2.4	3.4	33.1	21.2	43.2	92	69
10	Skämningsfors	2.6	2.6	18.7	42.7	36.0	64	39
11	Bondberget	2.2	5.0	29.2	16.8	51.8	73	40
12	Ingaryd			62.5	25.0	12.5	100	38
13	Borsaskögle	4.3	4.3	31.9	21.3	42.6	83	55
14	Bollbynäs	14.2	14.2	44.9	22.4	18.4	80	76
15	Smala betet	10.0	35.7	50.0	30.0	10.0	30	0
16	Omberg 0	1.2	1.2	9.3	12.8	76.6	60	26
17	Omberg 1	1.3	5.1	5.3	11.8	81.6	54	33
	Total	1.8	2.6	16.0	22.5	59.8	74	44

[1] only ash >1.3 m height.

3.1.2 The distribution of ash in plot inventory; health class vs. height class

Top height class distribution by health class (figure 4, table 6): The tendency in the entire plot inventory was that the frequency of height class 6 (>20 m) decreased with increasing health class (i.e. decreasing health) (figure 4). This tendency was confirmed in 3 out of 8 analysed sites as a significant difference in the distribution of the different health classes in the top height class (p-values in table 6). In two of the analysed sites (no. 1 and 16) the only difference in the top and lower height class distribution was more ash in health class 4 in the top height class and less ash in health class 4 in the lower height classes. Health class 4 represents ash with more than 65 % visible crown loss. Sites no. 9, 11 and 17 showed no difference in the top height class distribution in the different health classes (see table 6). In the total plot inventory there is a significant difference ($p<0.0001$) in the distribution of the different health classes in the top height class.

Canopy tree distribution by health class (figure 4, table 6): Height class 5 and 6 (in three sites height class 4 and 5) are representing the majority of ash trees in the canopy of the plot inventory sites. In the entire plot inventory the frequency of the lower canopy ash (height class 5) did not show the same tendency as the top canopy trees (height class 6), since the lower canopy distribution increased in health class 4 (see figure 4). The canopy tree distribution in the different health classes, were compared to the lower height classes (see table 6). In the entire plot inventory and in 7 out of 10 sites there was a significant difference in height class distribution among health classes mostly due to more ash in health class 4 and less ash in health class 5 in the canopy trees (p-values in table 6). In sites no. 9, 13 and 17 there were no difference in health class distribution in the canopy trees compared to the lower height classes.

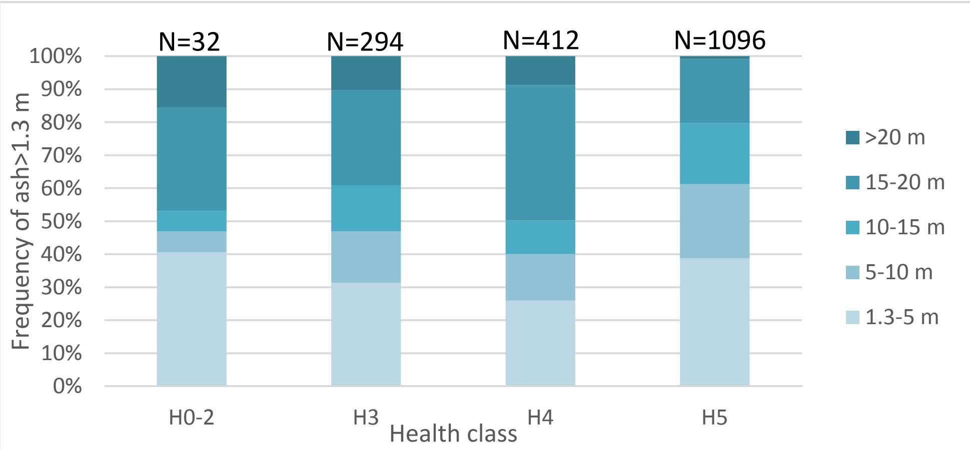

Figure 4. The distribution of height class for each health class in the total plot inventory. Each bar represents the total amount of ash in each health class.

Diameter at breast height and crown class displayed the same distribution as height class for the different health classes.

Table 6. *Chi square test in different sites in the plot inventory, on the distribution of top one and top two height class compared to the other height classes in different health classes. Since analysis including healthier ash was invalid in most sites, analysis excluding healthier ash was also performed. H0-5 is health class 0-2; 3, 4 and 5. H3-5 means health class 3, 4 and 5. Significance level was set to 0.05. P-values below this were considered as significant and marked with an asterisk.*

		Difference in top height class		Difference in top 2 height classes	
Site Nr	Site name	H0-5	H3-5	H0-5	H3-5
1	Västergården		0.004*		0.01*
5	Strands raviner	<0.0001*	<0.0001*	<0.0001*	<0.0001*
8	Ost Lundtorp		0.013*		<0.0001*
9	Dintestorp		0.69		0.167
10	Skämningsfors		<0.0001*		<0.0001*
11	Bondberget		0.185		<0.0001*
14	Bollbynäs			0.016*	0.008*
13	Borsaskögle				0.155
17	Omberg 1		0.125		0.248
16	Omberg 0	0.008*	0.003*	0.024*	0.009*
	All ash in plots	<0.0001*	<0.0001*	<0.0001*	<0.0001*

3.2 Sites of less dense structure- Inventory of all ash

In this inventory the criteria for establishment of plots was not fulfilled due to more space in between the trees and hardly any undergrowth. The mean values of height class and dbh revealed that these surveyed ash trees were considerably larger all together (see mean size values in table 7) compared to the plot inventory sites (table 4) with the exception of site no. 18, Pilalandet, a seed stand with ash trees of considerable height (mean height class 5.31) but without the same generous space in between the trees as in the other sites in this inventory, hence the lower mean dbh. A substantial proportion of the ash trees in this inventory were considerably older (no data, only visual conclusion), compared to ash in the plot inventory, especially in sites no. 10 (avenue), 11 (pasture land), 12 (avenue/pasture land) and some of the ash in site no. 19 (pasture land). These three sites were also selected from data on valuable trees, meaning old and strongly built trees, provided by the County Administrative Board.

Healthier ash (health classes 0-2) were detected in all sites although some of them had small sample sizes, like site no. 10 (n=12). All sites in this inventory had a mean health value (i.e. mean crown dieback value) below 4.0 while the sites in the plot inventory generally had a mean health value above 4.0. The number of trees surveyed in each site were generally less in this inventory compared to the plot inventory sites, except for site no. 18.

Table 7. *Number of ash in the healthier classes (H0-2) and mean values from all ash in each site in the inventory of all ash.*

Site No.	Site name	Measured/ assessed ash	No. of ash in health class 0-2 in site	Mean health class (SE mean)	Mean height class (SE mean)	Mean dbh (cm) (SE mean)
10	Skämningsfors avenue	12	2	3.25 (0.28)	5.58 (0.15)	83.16 (6.15)
11	Bondberget pastured	24	6	3.25 (0.21)	5.75 (0.09)	61.99 (3.86)
12	Ingaryd avenue/ pastured	36	3	3.78 (0.14)	5.81 (0.08)	71.53 (4.30)
18	Pilalandet	89	6	3.99 (0.1)	5.31 (0.07)	22.98 (1.09)
19	Lövhult	20	4	3.50 (0.25)	4.85 (0.20)	48.77 (7.16)
	Total	181	21	3.70 (0.08)	5.43 (0.05)	45.62 (2.25)

3.2.1 Distribution of health class in sites- inventory of all ash

In this inventory, 12 % of the total number of ash were considered healthier (in health classes 0-2). Within this health group merely 1.7 % (3 out of 181 ash trees) had visible crown dieback less than 12.5 % (health class 1) and constituted the healthiest part of the group. Compared to the total frequency in the plot inventory (table 5), these sites together had a greater proportion of healthier ash (see table 8). Generally, individual sites had a higher proportion of healthier ash compared to the sites comprising the plot inventory, with the exception of sites no. 12 and 18 which had 2 sites out of 17 in the plot inventory with a higher proportion of healthier ash. Although including the healthier ash found outside plots in the sites of the plot inventory, only 4 sites out of 17 had higher proportion than the site of lowest proportion of healthier ash (no. 18) in the inventory of all ash.

Health classes 4 and 5 together, representing ash with more than 65 % visible crown dieback (based on class mean, see table 3), varied between 45 and 74 % among sites and was 64 % for the entire inventory of all ash (3 out of 5 ash trees). Unlike the plot inventory, health class 4 had the greatest proportion of this combined health group, with the exception of site no. 19. In this site, in contrast to the others, the highest proportion of ash was in health class 3.

The proportion of dead and almost dead trees was generally substantially lower in the sites in this inventory compared to the sites in the plot inventory. In the inventory of all ash, the site with the highest proportion of dead and almost dead ash

(33 %), still had a considerably lower proportion than the majority of the sites in the plot inventory. In the entire inventory of all ash the total proportion of dead or almost dead ash was 23 % (table 8); less than half of the same proportion in the entire plot inventory (60 %).

Table 8. *Frequency of ash in health class groups and size groups in the different sites in the inventory of all ash (plot inventory not possible).*

		Frequency of ash in health class H (%)				Frequency of ash in size groups (%)	
Site No.	**Site name**	**H0-2**	**H3**	**H4**	**H5**	**Codominant/Dominant**	**Dbh 35-130 cm**
10	Skämningsfors avenue (n=12)	17	33	50	0	92	100
11	Bondberget pastured (n=24)	25	29	38	8	100	92
12	Ingaryd avenue/ pastured (n=36)	8	22	53	17	94	92
18	Pilalandet (n=89)	7	19	41	33	36	11
19	Lövhult (n=20)	20	35	20	25	50	40
	Total (n=181)	12	24	41	23	61	47

The frequencies of ash in different size groups, in each site (table 8), indicate the variability in forest structure among the sites. The total frequency of codominant/dominant ash was 61 %, compared to 26 % in the plot inventory. The total frequency of large diameter ash >35 cm dbh was 47 %, compared to 4 % in the plot inventory.

3.2.2 Distribution of ash in inventory of all ash; health class vs. height class

The distribution of height class in each health class, showed a clear tendency for the top height class (>20 m) to decrease with increasing health class (i.e. increased severity of crown dieback) (figure 5) while height class 5 (15-20 m) displayed the opposite trend, an increase with increasing health class. A weak similar tendency was seen in height class 4 (10-15 m). Diameter at breast height and crown class displayed the same distribution as height class for each health class.

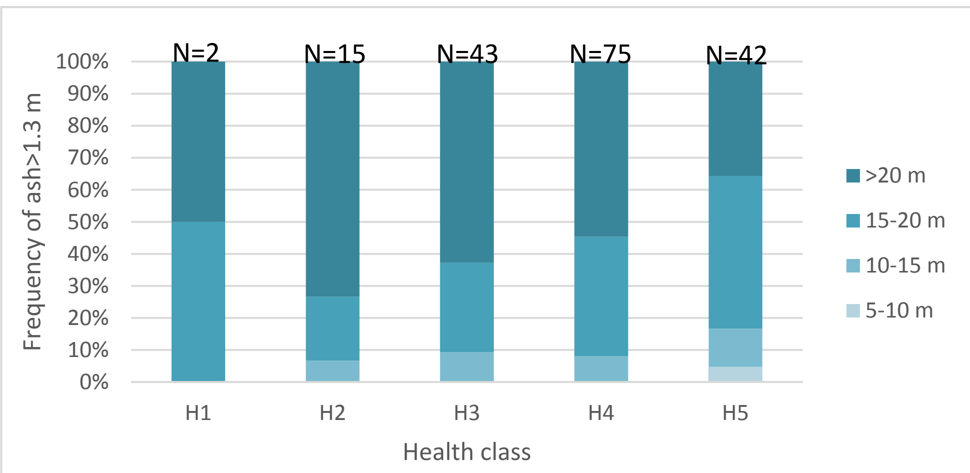

Figure 5. The distribution of height class in each health class in the total inventory of all ash. Each bar represents the total amount of ash in each health class.

3.3 Healthier ash identified in all sites (within plots and outside)

The search for healthier ash in all visited sites resulted in a total of 69 trees registered, two in the supreme health class (0), nine which had less than 12.5 % visible crown dieback (health class 1, see table 3) and 58 that had less than 35 % visible crown dieback (health class 2). These healthier ash trees were distributed unequally in the sites according to Table 4 and Table 7. Since no ash less than 10 m height was surveyed outside plots and the inventory of all ash consisted of 1 % (2 out of 181) ash less than 10 m, the height distribution of the healthier ash was only interesting in the plot inventory. The number of healthier ash below 10 m equals the number of healthier ash more than 15 m of height, while in the total plot inventory the proportion ash less than 10 m was 54 % and the corresponding proportion of ash higher than 15 m was 30 %.

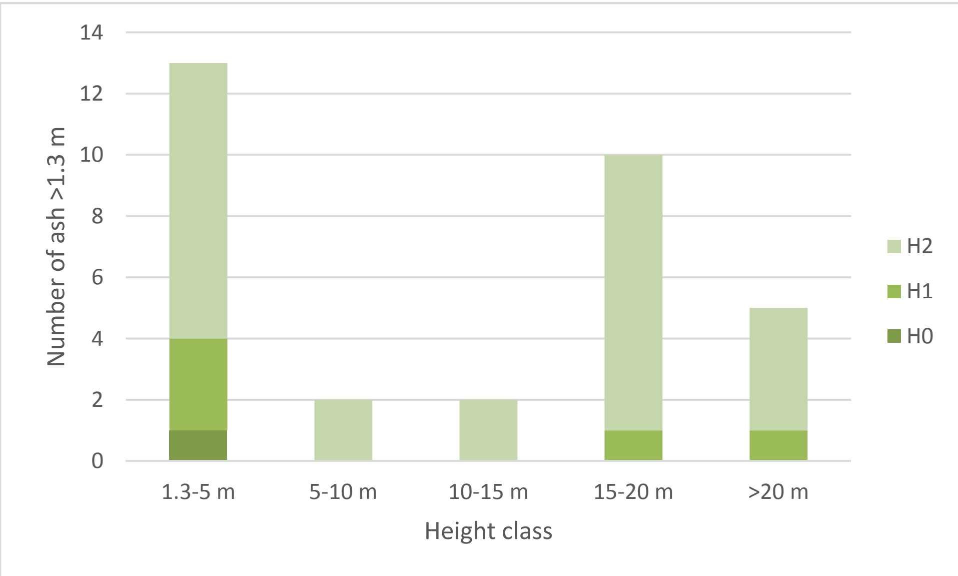

Figure 6. The number of healthier ash (H 0-2) in different height classes in the plot inventory.

3.4 Analyses of plot inventory

3.4.1 Distribution of healthier ash in size groups

Of the 17 sites in the plot inventory, 11 had ash trees within plots in health class 0-2. To examine if healthier ash are distinguished from the dead and almost dead ash in size, a Fisher’s exact test was conducted comparing the following size groups: crown class, height class and dbh between health classes 0-2 and 5. The size groups were separated to compare the canopy trees to the understorey trees. One site, Smala betet, was unfit to analyse in this way, due to both a small sample size (n=10) and all ash trees being in larger size groups (i.e. height class >15 m).

<u>Crown class:</u> The majority of sites showed no difference in the distribution of crown class between healthier and dead/ almost dead ash. Two out of 10 had significantly higher proportion of healthier codominant/dominant ash (see table 9). In the total plot inventory healthier ash had a significantly higher proportion of trees in crown classes codominant/dominant (see table 9).

<u>Height class groups:</u> The majority of sites showed no difference in the distribution of height class between healthier and dead/almost dead ash. Two out of 10 sites had significantly higher proportion of healthier ash in the canopy (height classes 15 to 20 and >20 meters) (see table 9). In the total plot inventory healthier ash had a significantly higher proportion of trees in the canopy (height classes 15 to 20 and >20 meters) (table 9).

<u>Dbh groups:</u> The majority of sites showed no difference in distribution of dbh groups between healthier and dead/ almost dead ash. Two out of 7 sites had significantly higher proportion of healthier ash with dbh >30 cm (see table 9). In the total plot inventory healthier ash had a significantly higher proportion of trees with diameter at breast height over 30 cm (table 9).

Table 9. *P-values of Fisher's exact test comparing health class 0-2 and health class 5 to different size groups. Significant level was set to 0.05. P-values below this were considered as significant and marked with an asterix. Crown class in groups codominant/dominant and suppressed/intermediate. Height class in groups height class 2-4 (1.3-15 m) and height class 5-6 (15->20 m). Dbh in groups 0-30 cm and 30-130 cm at breast height. The empty spaces in the dbh column is due to no ash with dbh >30 cm in those sites.*

Site No.	Site name	Crown class	Height class	Dbh groups
1	Västergården	1.0	1.0	1.0
5	Strands raviner	<0.0001*	<0.0001*	<0.0001*
7	Sutarp	0.46	1.0	1.0
9	Dintestorp	1.0	1.0	1.0
10	Skämningsfors	0.015*	0.015*	0.0025*
11	Bondberget	0.067	0.067	0.29
13	Borsaskögle	1.0	1.0	
14	Bollbynäs	1.0	1.0	1.0
16	Omberg 0	0.1	1.0	
17	Omberg 1	1.0	0.35	
Total plot inventory		<0.0001	0.0005	<0.0001

In summary, for most sites there was no significant difference between healthier and dead/ almost dead ash in the different size groups. In the total plot inventory there was a significantly higher proportion of healthier ash in the larger size classes.

One of the sites (no. 5) that had a significantly higher proportion of healthier ash in the larger size groups had a large sample population (n=540) of the total plot inventory data (n=1834).

3.4.2 Associations in site variables

Variables from each of the sites in the plot inventory; mean values for health class, height class and dbh (table 4), number of measured/assessed ash trees (table 4), proportion ash in health class 5 (table 5) and proportion ash comprising the total species composition (table 1) were analysed for their association using a Spearman's ranked correlation test.

A weak positive correlation was found between the number of measured/assessed trees in sites and mean health value class (and proportion of ash in health class 5) (see table 10). So a small set of data somewhat associates with a lower mean health value class and in a larger set of data more unhealthy ash trees were found and consequently the mean health value class was higher.

Another quite weak negative correlation was found between the proportion ash in species composition and the proportion ash in health class 5 (and the mean health value class) (see table 10). So a higher proportion of ash in the species composition is somewhat associated to a lower proportion of dead/ almost dead ash.

No correlation was found between the mean health value and the mean values of height class and dbh respectively (table 10).

Table 10. *Spearman´s rank correlation coefficient measuring the association between site variables from table 1, 4 and 5. Significance level was set to 0.05. P-values below this value were considered significant and the interesting significant p-values are marked with bold numbers.*

Spearman Rho P-value	Mean value height class	Mean value dbh	No. of ash trees measured/assessed	Proportion ash in health class 5	Proportion ash in species composition
Mean value health class	0.100 0.701	-0.113 0.667	0.538 **0.026**	0.950 0.000	-0.484 **0.049**
Mean value height class		0.868 0.000	- 0.179 0.492	0.069 0.793	-0.336 0.187
Mean value dbh			-0.299 0.243	-0.152 0.559	-0.233 0.368
No. of trees measured/assessed				0.531 **0.028**	0.104 0.690

Proportion ash in health class 5	-0.508 **0.037**

3.4.3 Distribution of health class and dbh in ash compared to elm

In six sites in the plot inventory *Ulmus glabra* was in top three in the share of species composition. The frequency of health classes and dbh of *F.excelsior* and *Ulmus glabra* in these sites is shown in figure 7 in the appendix. In all of these six sites *U. glabra* had significantly higher proportion of healthier trees compared to ash. The distribution of dbh between *U. glabra* and *F. excelsior* was significantly different in 3 out of 4 sites where it was possible to do these analyses. This difference in dbh distribution between the two tree species was attributed to; more elm with dbh between 10 to 20 cm (site no. 5); more elm with dbh between 5 to 10 cm (site no. 8); and more elm with dbh less than 5 cm (site no. 16).

To examine if the health class distribution in *U. glabra* differed between the lower (<10 m) and the top (10 m to >20 m) height classes, an analysis was performed comparing the extreme health classes (H0-2), (H4-5) and the intermediate health class (H3), which represents trees with a visible crown dieback between 35 % and 55 %. The results showed no difference in this distribution in 3 out of 5 sites (where analyse was possible) and the difference in the other two sites was mostly due to more elm in health class 3 in the top height classes. When health class 3 was removed from the analysis and only healthier and unhealthy elm distribution in top and lower height classes were compared, there was no difference in 4 of 5 sites. In the remaining site a small but significant difference in the distribution was evident with somewhat fewer unhealthy elm trees in the top height classes (10 m to >20 m).

Table 11. *P-values from Chi square test comparing differences in distribution in health class and dbh between F. excelsior and U. glabra and the health class distribution for the elm trees in the lower (<10 m) and top (10 m to>20 m) height classes. Significance level was set to 0.05, P-values below this were considered significant and marked with an asterisk. (F) means the p-value was acquired from Fischer's exact test.*

		Fraxinus vs.Ulmus glabra		Health class distribution Ulmus glabra	
Site no.	Site name	Health class	Dbh	H0-2;H3;H4-5	H0-2,H4-5
2	Västra gröndalen	<0.0001*		0.048*	0.038*
4	Fäktahål	<0.0001*	0.63		0.38 (F)
5	Strands raviner	<0.0001*	<0.0001*	0.32[1]	0.32
8	Ost Lundtorp	<0.0001*	0.013*	0.014*	0.07
13	Borsaskögle	<0.0001*		0.13[2]	
16	Omberg 0	<0.0001*	<0.0001*	0.74	0.44

[1] No elm trees in health class 3.
[2] No elm trees in health class 4-5.

4 Discussion

There is a low proportion of healthier ash in the entire inventory. The question is whether these healthier trees are more resistant to ash dieback or not. This study has not examined the existence of *H. fraxineus* in the unhealthy trees (by isolation / biological detection), instead an assessment of visible crown dieback was conducted. Assessed health status (i.e. level of damage) was shown by McKinney *et al.* (2011) to strongly correlate with the incidence of necrosis and was considered by the authors as a dependable way to estimate *H. fraxineus* deterioration of ash trees. Also Bengtsson *et al.* (2014) found a negative (albeit weak) correlation between necrosis growth and assessed tree health on ash. To be able to assume that a healthier ash is more resistant, it is important to exclude the possibility of disease escape. If a healthy ash is surrounded by sick or dead trees, there is high likelihood that the particular tree has had equal probability of exposure to airborne inoculum, but somehow managed to stay healthy, possibly due to active or passive defences (Johansson *et al.*, 2009). Yes, it could be due to other causes, like other antagonistic or hyper parasitic fungi or microorganisms on that specific tree, similar to human flora of bacteria that can protect us from aggressive bacteria. However this hypothesis is partly contradicted by Bakys *et al.* (2008) who found comparable combinations of fungal species on both symptom free shoots of ash and on those with various symptoms. Another explanation for the variation in disease expression could derive from differences in virulence among *H. fraxineus* genotypes (Kraj & Kowalski, 2014) though the abundant spread of ascospores prevent this from explaining the difference in health within the same area. Instead several studies of clones have shown evidence of genetic variation in the susceptibility of ash to *H. fraxineus*, where some clones show signs of tolerance (Stener, 2013; McKinney *et al.*, 2011; Stener, 2007) even when the pathogen was inoculated (McKinney *et al.*, 2012). Since unhealthy trees existed and prevalently dominated in all of the sites in the survey herein, all ash have probably been heavily exposed to the pathogen and hence make the assumption of true resistance in the healthier ash possible.

The low proportion of more resistant trees identified in this inventory is in agreement with other studies both in the aspect of low proportion of healthier trees (Lobo *et al.*, 2014; Kjaer *et al.*, 2012) and also in the aspect of limited levels of resistance (Lobo *et al.*, 2014; Stener, 2013; McKinney *et al.*, 2011). Hardly any trees appear to be completely resistant, instead some of them are probably partially resistant and this is consistent with the study of Stener (2013) and also the survey of 101 mother trees by Kjaer *et al.* (2012) where only one of the mother trees produced almost healthy offspring and four of the mother trees had progenies moderately healthy after three years. In the survey herein, searching through every visited site, only two almost completely healthy (visible crown dieback 0-4.2 %) ash trees were found and nine with visible crown dieback less than 12.5 %. The rest of the healthier ash trees found had visible crown dieback between 12.5 % and 35 % and are hence quite largely affected by ash dieback. Assessing health is crucial both in the aspect of subjectivity and also how the classes of health are translated. In contrast to the survey herein, in a study by Bengtsson (2014) an ash tree with more than 30 % of the crown affected was labelled seriously affected. Ensuing this categorization a fundamental part of the healthier ash in the survey herein is labelled wrong. However, studying examples of assessed ash in the survey by Wulff and Hansson (2011), an ash crown determined as thinned out 20 % would in my subjective assessment not have been in the range of healthier classes. The conclusion of this suggests comparing the assessed results from various surveys to be somewhat hazardous but that the amount of more resistant ash in this study is definitely low. Moreover, these discrepancies indicate the need for a more standardized rating system when assessing and classifying crown damage on trees.

A source of error found too late in this survey was a misjudgement of how the rating system by Kirisits and Freinschlag (2012) could be registered. This results in a somewhat insecurity in the ash trees categorized in health class 1; some few trees in this group might actually belong to health class 2. Since health class 2 also is classified as healthier, this mistake is negligible in the results.

There are some small but interesting differences between the plot inventory and the inventory of all ash in respect to the proportion of healthier ash. In the plot inventory the low proportion of healthier ash (2 %) was not increased much when healthier ash found outside plots were included (2.6 %). The ash trees in avenues, in pasture land and in one of the seed stands (inventory of all ash), with more open forest canopy structure, have somewhat higher proportion (12 %) of healthier ash. This can either be due to an assessment failure; the lack of competition in light and water/nutrition result in better assessed health compared to dense forest ash that for instance might lose branches due to shade, not due to pathogens. Alternatively, this higher proportion of healthier ash in open forest canopy structure might be due to less competition of light, water and nutrition contributing to better vigour to sustain pathogen infections. Bakys *et al.* (2013) did not find any clear relationship between stand density and the incidence of ash decline, although the unthinned plots were more affected compared to the thinned plots. Instead some ash dieback symptoms increased with decreasing density (ibid.) Since ash demands more light with age and insufficient light conditions increase ash mortality (Dobrowolska *et al.*, 2011) it seems logical that shaded ash trees in dense forests could be more stressed and hence more susceptible to various diseases. Competition of nutrition and water could also influence the capacity to fight off pathogen infections. This has been seen in other species, like *Pinus* being more susceptible to *Gremmeniella* in dense stands (Niemelä *et al.*, 1992) and the decreasing ability for active defence with increased competition in crops (Karban *et al.*, 1989). Skovsgaard *et al.* (2010) found a relation between subordinate growth and ash decline in a young ash stand and connected this to reduced vitality in the smaller trees. A weak positive correlation between health and size in ash was found by Bengtsson *et al.* (2014) and that only larger trees were completely healthy. An interesting anecdote is site no. 14, dominated by a dense colony of shrubs (76 % with dbh <5 cm) but with hardly any overstorey. The lower mean value of health (i.e. lower visible crown dieback) of this site suggests that it could be the canopy competition and not the competition within shrubs that play a role in the susceptibility to ash decline. This could also explain the fact that there was no difference in this site between the canopy trees and the understorey trees in distribution of healthier and dead trees respectively. But since the main part of the plot inventory sites showed no difference in the size distribution between healthier and the dead and almost dead ash, this suggests that there generally are no differences in susceptibility between canopy trees and understorey trees. There was however a greater proportion of healthier ash in the canopy compared to the dead/almost dead ash in two of the sites and also in the total number of trees in the plot inventory. This difference in the total plot inventory is probably strongly influenced by one of the sites (no. 5) with a very large dataset (n=540) out of the total number of trees in the plot inventory (n=1834). Additionally, if the analysis included ash with visible crown dieback between 35 and 82 %, the majority of sites had significantly higher proportion of canopy ash trees with visible crown dieback between 65 and 82 %. This group of ash is only in a marginally better condition than the dead/almost dead ash and will probably die within the next few years. This prediction is supported both by the results by Lobo *et al.* (2014) where the survival rate merely was 34 % for the ash trees with more than 50 % crown dieback five years earlier and also by the fact that 10 % of the ash trees have died per year in Lithuania (2009-2013) (Lygis, personal communication)[2]. However, some sites (3 out of 8) had significantly less dead/almost dead

2. Vaidotas Lygis, PhD, Institute of Botany of Nature Research Centre, 2014-12-04

ash in the top height class. These trees also dominate the canopy as well as the understorey and ought to have an advantage from this. But in 5 of 8 sites there were no difference between top and lower height classes or merely a larger distribution of top-canopy ash with more than 65 % crown dieback (health class 4). Summarizing all these results, the conclusion is that there are exceptions where at least the greater trees of the canopy seem to be less susceptible than the understorey trees but that generally ash trees of all sizes and forest positions show the same disability/ability for some resistance. An alternative conclusion is that the greater trees merely have a slower disease progression which have been noted in some studies in greater/older trees (Keßler, 2012; Kirisits & Freinschlag, 2012).

The fact that *Fraxinus excelsior* is listed as endangered and *Ulmus glabra* as critically endangered on the Red list of endangered species in Sweden (Sandström *et al.*, 2015) suggests elm to be more threatened by Dutch elm disease than ash is to ash decline. However, *Ulmus glabra* has a significantly higher proportion of healthier trees compared to ash in all six of the compared sites. Dutch elm disease primarily affect the larger elm trees (Peterken & Mountford, 1998), mainly leaving persistent elm stands under the height of 8 m (Brunet *et al.*, 2014). Due to this a difference in health class distribution of *Ulmus glabra* between the lower (1.3 - 10 m) and top (10 to >20 m) height classes was expected but in the majority of sites there was no difference. In the sites that did show a difference it was surprisingly due to fewer unhealthy elm trees in the top height classes (comparing unhealthy and healthy) or differences in elm with visible crown dieback between 35 and 55 % (including this health class in the comparison). When comparing the difference between ash and elm among dbh classes in four sites, elm had a significantly higher proportion of smaller trees (dbh < 5 cm (shrubs?)) in one site and more trees with dbh between 5 to 10 cm (shrubs?) in another site (only 4 out of 6 sites had valid results). Studying the frequency of dbh (figure 7 in appendix) of these six sites, it was clear that the elm trees dominantly consist of smaller trees (shrubs?) in at least half of the sites. Whether elm was affected previously in this area and this is the remaining survivors or if this area escaped Dutch elm disease can only be speculated. Still, the current state in these sites suggests that ash is more threatened by ash dieback than elm by Dutch elm disease which is inconsistent with the current ranking on the Red list of endangered species. Hence, if the current situation in these surveyed sites is illustrating the situation in Sweden, a reinvestigation of the category of *Fraxinus excelsior* on the Red list of endangered species could be plausible.

There was a massive amount of unhealthy ash in this survey. In the plot inventory 60 % were dead or almost dead and more than 20 % had visible crown dieback between 65 and 82 % (health class 4). This combined group of seriously affected ash will almost certainly not recover (Lobo *et al.*, 2014)(also see H4 and H5 in figure 3) and four out of five ash trees (82 %) in the plot inventory are in the state of health with visible crown dieback 65 to 100 %. There was also an association between more measured/assessed ash trees and higher value of health class mean (i.e. more visible crown dieback), concluding that the more ash that was surveyed, the more unhealthy ash was found. Three out of five ash trees (64 %) were seriously affected (visible crown dieback 65-100 %) in the inventory of avenues, pasture land and a seed stand (inventory of all ash) though, in contrast to the plot inventory, with the majority (41 %) with visible crown dieback between 65 and 82 % (health class 4) and the remainder (23 %) dead and almost dead. Consequently this suggests a difference in susceptibility between the groups of ash where the more susceptible (plot inventory) mainly consists of smaller, younger ash in dense forests compared to the somewhat less susceptible group (inventory of all ash) of larger ash trees with probably less competition in light as well as water and nutrition. Since 44 % of the ash in the plot inventory had a diameter at breast height of less than five cm, I would expect a proportion of those trees to die naturally, even without the occurrence of *Hymenoscyphus fraxineus*. However, the light demanding species *Prunus avium*, with the majority of trees in shrub size in site no. 16, had a proportion of unhealthy trees merely consisting of 7 % (n=177), this probably representing trees naturally outrivalled (see figure ? in appendix?). Additionally, no correlation between the mean values of health and the mean values of height and diameter at breast height respectively was found comparing the different sites in the plot inventory. So sites dominated by shrubs did not generally have a higher mean value of health (i.e. more visible crown dieback) compared to sites with shrubs in minority. Conclusively, this difference in proportion unhealthy ash might be marginal, since probably none of these ash trees will recover. Still it seems to be a somewhat delayed process of dying in the inventory of avenues, pasture land and seed stand, compared to the plot inventory, which is consistent with the earlier mentioned surveys of a slower disease progress of greater/older trees (Keßler, 2012; Kirisits & Freinschlag, 2012).

The weak negative correlation between the proportion ash in species composition and the proportion dead/almost dead ash found in the plot inventory implies that the more pure ash stands would have less seriously diseased ash trees compared to the more mixed stands. In the survey of ash by Wulff and Hansson (2011) comparing stands with the major part ash to stands with less than half the share ash, there were a larger portion of dead ash in stands with a major part of ash but a smaller portion of ash with crown dieback between 60 to 99 %. The mixture of species are often beneficial to health in pathogen affected trees, especially when the pathogen is dispersed by wind where the species admixture obstruct spore diffusion (Pautasso *et al.*, 2005). The correlation found herein was weak and could actually be due to other factors, like the constitution of soil and water, but it concludes that the ascospores of *H fraxineus* are abundant everywhere and that no ash in this area can escape their propagation.

Hymenoscyphus fraxineus arrived to Sweden in 2001 (Johansson *et al.*, 2009) and in the current situation, according to this survey, the pathogen is affecting almost all and killing the majority of ash populations in the studied areas. This tree species is of great importance culturally, environmentally and also somewhat economically despite its low prevalence, but the future role for ash seems very insecure due to ash dieback (McKinney *et al.*, 2014; Kirisits & Freinschlag, 2012). In Lithuania, ash has been affected by the disease at least an additional 5-6 years compared to Sweden and a large portion of the ash stands have consequently been diminished. Visiting some of the Lithuanian ash forests a month after conducting the survey in Sweden, highlighted a great difference between our countries; in this survey there was a large proportion of unhealthy ash but in the Lithuanian forests the ash trees were generally missing. Almost all of the visited ash stands in Lithuania were presently former ash stands with only residues left in the form of diseased seed trees and a few remaining ash seedlings. Harvesting of diseased/dead ash clearly contributed to this picture of Lithuanian former ash stands. However, the conclusion is that with the results from the surveys presented herein, with almost all ash seriously affected by ash dieback, this picture from Lithuania might represent the future prospects of ash in this area of Sweden. Consequently, within years only residues of ash might remain on these sites and other species will instead dominate.

In total, 69 relatively healthy ash trees were found in total in the surveyed sites which results in to an average of almost five ash trees per adjacent area. There could be more resistant ash in the range of these trees pollen movement but since ash constitutes a very small portion of the standing volume in this area, the chance of this is limited. Instead there is a major risk that not even these healthier trees will show sufficient resistance and hence leave even less surviving ash in the pollen range of each other.

Some landowners in the survey herein have referred to a health improvement in some few ash trees, suggesting they may recover. This phenomena is mentioned in some studies (Bengtsson, 2014; Stener, 2013) and also by Lobo *et al.* (2014) who detected a crown enhancement in progenies of some mother trees. Indeed this could happen as a result of epicormic shoots making the crown appear healthier (Bengtsson, 2014) or as a consequence of a defence system in a more resistant ash conquering the pathogen? Many studies put their hope to the more resistant ash trees (Pautasso *et al.*, 2013; Stener, 2013; Kjaer *et al.*, 2012) and since the trait of more resistance is highly heritable (Lobo *et al.*, 2014; Kjaer *et al.*, 2012; Pliura *et* *al.*, 2011) this provides a potential to exploit in a breeding program. Further clarification is needed about the mechanisms of more resistance and the attributed quantitative genetics (McKinney *et al.*, 2014). The additional aim of this thesis was to locate healthier candidates of ash for potential use in a breeding program. Some of the trees (25 ash trees) identified as healthier in this survey have already been selected for scion collection and will, with others, be resistance tested further. This work together with other research about the concept of more resistance will hopefully result in a successful preservation of common ash. The magnitude of dying and dead ash in this survey reveal the necessity of such actions. If surviving ash is left as islands in the landscape, the genetic variation of ash will be depleted. Therefore there is a need to build up a gene bank of resistant ash, to preserve and in time maintain the current level of genetic variation, which hopefully in the future, will secure the species ability to cope with future challenges. Another threat approaching is the emerald ash borer (McKinney *et al.*, 2014) and there are arguments for breeding a large collection of more resistant trees due to this imminent risk as well as other potential threats (McKinney *et al.*, 2014; Pautasso *et al.*, 2013; Kjaer *et al.*, 2012).

Another potential prospect for ash is a mutation of a mitovirus on *Hymenoscyphus fraxineus* causing hypo virulence in the pathogen. Such progress would need to occur and thereafter disseminate hastily since almost all ash in the survey herein are already affected by the disease. To hope for that is one option but an active effort such as a breeding program or development of possible counteractions would be more in line with the current situation, considering the evidence that ash is in such a serious state of declining health.

Conclusions:

- The great majority of the ash population in this area seem to be highly susceptible to ash decline and will most probably die within a few years.
- The proportion of more resistant ash is very small and scattered in this area and all facts point to a probable risk of further loss of genetic diversity to genetic drift and inbreeding.
- Genetic control through a breeding program does not only provide the means to sustain as much genetic variation as possible, it can also serve as an opportunity to learn more about the mechanisms associated with resistance and how those should be selected for.
- No clear associations were found between size and more resistance or high susceptibility.

- In these surveyed sites, ash is more threatened to ash decline than elm is to Dutch elm disease and if this situation is representative for the whole of Sweden, this indicates the need for assigning a higher threatened status for ash on the Red list of endangered species.

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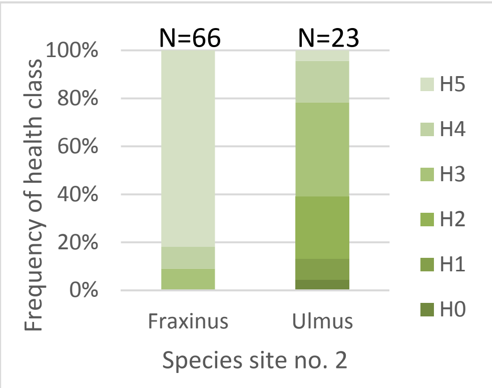
6 Appendix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Frequency of health class
100%
80%
60%
40%
20%
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Fraxinus
Ulmus
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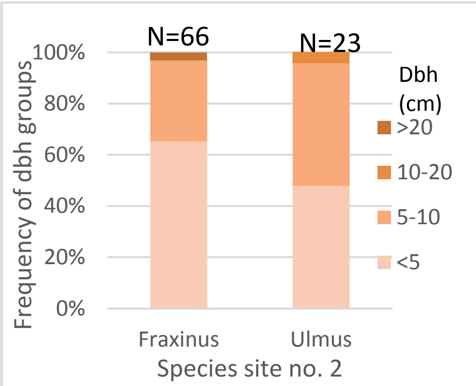
H5
H4
H3
H2
H1
H0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Ulmus
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Dbh (cm)
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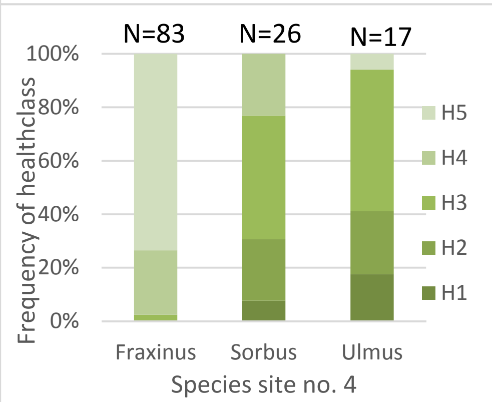
10-20
5-10
<5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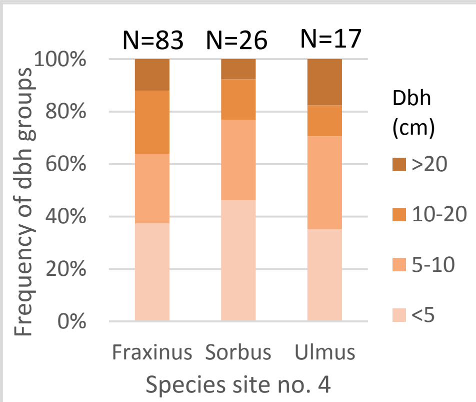
Sorbus
Ulmus
Species site no. 4
H5
H4
H3
H2
H1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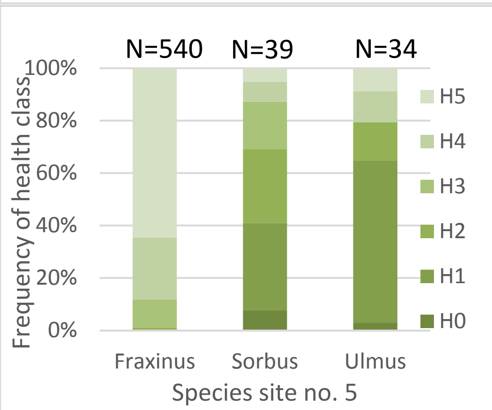
N=26
N=17
Frequency of dbh groups
100%
80%
60%
40%
20%
0%
Fraxinus
Sorbus
Ulmus
Species site no. 4
Dbh (cm)
>20
10-20
5-10
<5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Ulmus
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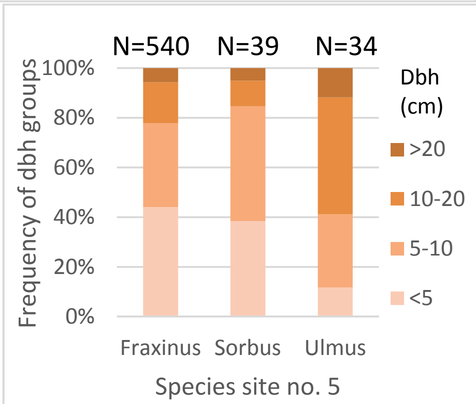
H5
H4
H3
H2
H1
H0


N=540
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Frequency of dbh groups
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80%
60%
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20%
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Fraxinus
Sorbus
Ulmus
Species site no. 5
Dbh (cm)
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10-20
5-10
<5

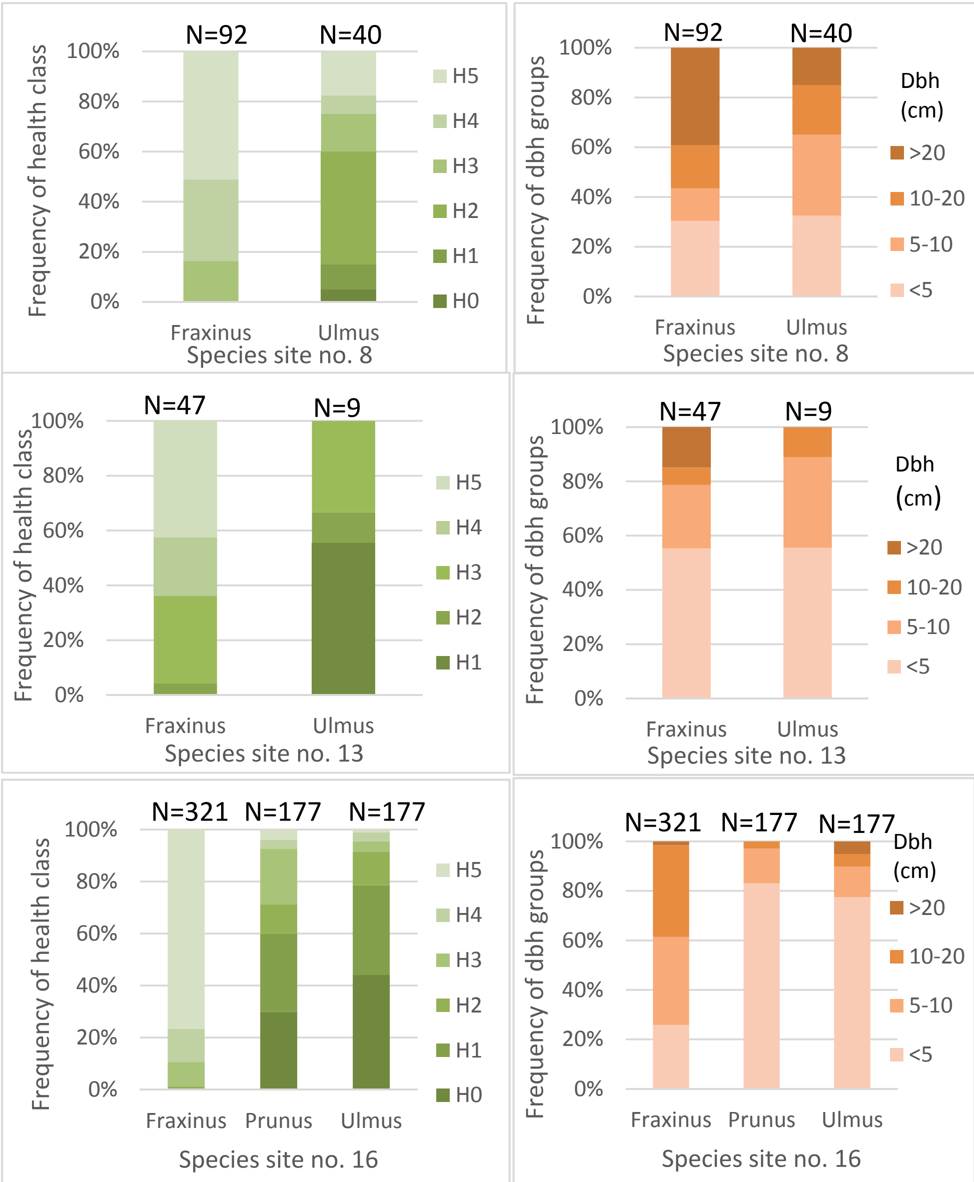

Figure 7. Comparing the distribution of healthclass and dbh of Fraxinus, Ulmus glabra and other species in six different sites.