The impact of ash dieback on ash regeneration in the forest reserve Dalby Söderskog

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Abstract

Ash dieback is affecting European ash (*Fraxinus excelsior*) throughout its distribution area, threatening economical forests and associated biodiversity. Studying regeneration is important to foresee the future development of ash. Dalby Söderskog forest reserve in Sweden was selected as a case study to investigate the impact of ash dieback on ash regeneration. Regeneration was sampled in 74 permanent sample plots recording two height classes (<1.3 m and ≥1.3 m, until 10 cm DBH) and five damage classes (healthy, browsed, slightly affected, severely affected, dead). Compared to studies in plantations or managed forests, the regeneration in Dalby natural forest is less damaged (20% of total regeneration, 50% of regeneration ≥1.3 m). The share of healthy small ash (<1.3 m) seems very high (77%), compared to only 5% for taller regeneration (≥1.3 m) indicating a reduced infection pressure for small ash. Canopy cover and damage class are significantly correlated (Chi-Square-Test) and can be described by a log-linear regression model for regeneration ≥1.3 m: An increase in canopy cover percentage results in decreasing density of severely affected ash regeneration. A low correlation coefficient and a low R^2 (0.15) suggest more influencing factors on damage to ash regeneration. Further research is needed to understand and predict the effects of ash dieback. In Dalby forest, ash will most likely not persist as a major species with only 400 stems ha^{-1} of healthy regeneration (≥1.3 m until 9 cm DBH) despite the comparably low damage.

1. Introduction

1.1. Ash dieback

Background

Ash dieback is a lethal disease, widespread in Europe today and a serious threat for the European ash (*Fraxinus excelsior*) and subsequently the associated biodiversity (Pautasso et al. 2013). Dead shoots lead to leaf loss in the crown, reducing the vitality of the tree often until its death. To date no fully resistant ash tree has been documented. No clone free of damage or disease symptoms has been found (McKinney et al. 2012a, Pluira et al. 2011, Stener 2012). The forest management and wood industry are affected by losing timber and a valuable species. Additionally, ecosystem functions could be reduced by a loss of biodiversity (Pautasso et al. 2013). In the United Kingdom, Mitchell et al. (2014) found 44 species depending on ash and 62 highly associated, from which 69 are already of conservation concern. The lichens among them are especially threatened by ash dieback because of their low colonization rate (Ellis et al. 2012, Fedrowitz et al. 2012). During a 5-year case study in Estonia, 50% of the ash trees died from ash dieback resulting in the loss of one epiphyte species (*Pyrenula laevigata*) and a likely local extinction of 3 more species (*Chaenotheca phaeocephala, Cetrelia olivetorum,* and *Arthopyrenia cinereopruinosa*) in the near future (Lohmus & Rumel 2014).

Causing agent

The pathogen causing ash dieback is *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea)*. The native anamorph *Hymenoscyphus albidus* is already declining and threatened by extinction due to competition pressure of the invasive *H. pseudoalbidus* (McKinney et al. 2012a), which probably originates from East Asia. The fungus was documented in Japan (Zhao et al. 2012) and also in north-eastern China (Zheng & Zhuang 2013). The higher genetic diversity of the Japanese population supports this theory and further genetic analysis in Europe revealed a lack of population structure and signs of a founder effect indicating an introduction of this species. The host trees in Asia are *Fraxinus mandshurica* and *F. chinensis*, but the fungus is not pathogenic on them in their home range (Gross et al. 2014). However, slight symptoms of ash dieback were also found on Mandshurian Ash growing in Europe. One hypothesis says that the fungus might have been introduced to the western parts of the former Soviet Union with seeds and plants of *Fraxinus mandshurica* from East Asia (Drenkhan et al. 2014).

Spread of ash dieback

Ash dieback is spreading increasingly in Europe since the first reports from north-eastern Poland in 1992. Presently it can be found in almost the entire native range of its host tree. It reached Switzerland in 2008, migrating the distance of 1200 km in 16 years. Thus the annual dispersal rate is extremely fast with 75 km on average, but slower along the northern and southern distribution boundary of *Fraxinus excelsior*, e.g. 30 km per year in Norway (Gross et al. 2014). In Sweden, ash dieback was first observed in 2001 and had spread over the Swedish distribution area of ash within few years (Barklund 2005, Timmerman et al. 2011). Apart from the European ash, symptoms of dieback in Europe were observed on narrow-leaved ash (*Fraxinus angustifolia*, native to Southern Europe, North Africa and Western Asia), *Fraxinus nigra* and *Fraxinus pennsylvanica*, which are native to North-America (Pautasso et al. 2013, McKinney et al. 2014).

Biology of *Hymenoscyphus pseudoalbidus*

The sexual reproduction of the fungus occurs on ash petioles or small stems in the litter of the preceding year (Gross et al. 2012). The released ascopores are wind dispersed (1,500 ascospores per hour and apotheticum, NW-FVA 2011). The ascospores infect ash leaves during summer from June to September (Gross et al. 2014). They attach to the leaf's surface and no wound of the leaf is necessary to let the fungus germinate through epidermal cells, where the mycelium spreads into the healthy tissue (Bengtsson et al. 2014). This produces pseudosclerital plates which leads to a necrosis of the cambium, causing the typical dieback symptoms of the tree (Metzler et al. 2013). Field records as well as inoculation experiments demonstrate the high pathogenicity of the fungus (Schumacher et al. 2010, McKinney 2012b, Bakys 2009a).

Symptoms of ash dieback

Visible symptoms of affected ash trees are necrotic wounds on leaves, petioles and young shoots with a typical orange-brown to purple stain on the bark which is free of exudates (s. Figure 1). They cause dieback of shoots or wilting of leaves (if the shoot is affected after leaf flush), elongated cankers on stem and branches, followed by leaf loss and crown dieback (cp. Figure 4, page 16). Dying trees often produce epicormic branches resulting in a bushy appearance of the crown (s. Figure 1). Since multiple infections can occur on a single tree, the new shoots can even accelerate the dieback (Bengtsson et al. 2014, Gross et al. 2014). Affected trees are more likely to be attacked by secondary pests, e.g. root rot pathogens like *Armillaria* sp., which penetrate the cambium of the tree causing a stem necrosis and therefore accelerating dieback (Bakys et al. 2009b, Gross et al. 2014, Pliura et al. 2011, Skovsgaard et al. 2010, Metzler et al. 2013).

Figure 1: Symptoms of ash dieback; i) necrotic bark lesions and extensive bark necrosis with typical orange-brown stain (Kirisitis et al. 2012); ii) dieback of crown and growth of epicormic branches in Dalby Söderskog (photo by Maja Dietrich, 2013)

Susceptibility of ash dieback

Ash trees of all age classes and in various forest types, open landscapes and in urban areas are affected by ash dieback in addition to trees in nurseries (Bakys et al. 2009b, Metzler et al. 2013). Disease symptoms were initially more severe in younger stands, e.g. in Germany the highest infections rates were found in ash stands with an age below 20 years (Schumacher 2011). But after further surveys, stands of all ages were found to be affected by the pathogen (McKinney et al. 2011, Witzel & Metzler 2011). Positive correlations of tree size and health conditions have been surveyed several times showing that small trees are more seriously affected than bigger trees (Skovsgaard et al. 2010, Kirisits & Feinschlag 2012, Bengtsson et al. 2014). Trees sized below average and suppressed individuals in dense stands showed more distinct decline symptoms than dominant trees (Skovsgaard et al. 2010, Cech and Hoyer-Tomiczek 2007). They have a reduced ability to recover and die off like young trees (Metzler 2010), sometimes very rapidly after 2-3 years following the infection. For large dimensioned trees the damage progress can be delayed and at least shortly compensated by epicormic branches (Witzel & Metzler 2011), often the process seems to be chronic (Schumacher et al. 2010). However, old trees can also die quickly, frequently due to secondary pathogens like *Armillaria sp* (Kirisits & Feinschlag 2012). In a German case study 40 year old trees died after 3 – 5 years following an infection by the fungus and 8% of the stand was lost after three years (NW-FVA 2011). Severe damage was observed especially along streams and rivulets in Austria (Cech and

Hoyer-Tomiczek 2007). In Germany highest infection rates occur on wet soils or soils with an abundant organic content, whereas lower damage rates were found on terrestrial soils or sites with changing moisture (Schumacher 2011). Wet sites seem to intensify and accelerate the damage probably due to delayed litter decomposition and humid micro climate (Metzler 2010). Also small trees were more often found on waterlogged, heavy clay soils (Bengtsson et al. 2014). Therefore, site conditions might influence susceptibility more than tree size itself. In general, the disease progress on mature trees is slower than on seedlings or young trees, only few saplings survive (Gross et al. 2014).

Silvicultural management of ash dieback

Once the lethal disease is established in the forest, no management is known to stop the dieback. Pruning doesn't help against new infection. In any case the cut would have to be very deep into the wood and damage the tree. The general advice for forestry companies is to not plant ash trees. However, natural regeneration can be used. Selection for thinning should be done in summer to clearly evaluate the vitality and degree of damage. In case of high damage, a rapid regeneration should be focused as long as the crown canopy exists. In order not to lose valuable timber, rapid harvesting after infection is recommended (Metzler et al. 2013). On that account many trees were felled before knowing if they were resistant and could survive. Today the species is propagated to sustain more ash trees where possible, but many still have to be cut due to safety regulations, for example root rot destabilization of the tree and in addition falling branches of dying ash trees (Hein 2009).

Inheritable partial resistance

Only a few individuals can maintain a low level of damage and remain in relatively good health. This partial resistance was found to be genetically predetermined and inheritable, whereas environmental factors showed a low influence on damage (Kjær et al. 2012). Therefore this small fraction of partly tolerant genotypes could be used for resistance breeding (Pluria et al. 2011, Bakys et al. 2009a, Stener 2013, McKinney et al. 2011, Enderle et al. 2015). Less susceptible trees flush and shed their leaves earlier than average, which might prevent the fungus from growing into the stem (McKinney et al. 2011, Gross et al. 2014). However, this could not be confirmed by studies in Austria (Kirisits & Feinschlag 2012), indicating that instead of genetic determination leaf infection leads to earlier leaf shedding. The difference could be explained by climatic conditions resulting in varying disease cycles. Furthermore studies in Norway reveal a maximum pressure of ascospores between mid-July and mid-August, before the leaf senescence (Hietala et al. 2013). The variation of susceptibility might be partly explained by the trees reaction to phytotoxins produced by

Hymenoscyphus pseudoalbidus (Cleary et al. 2014). Studies by KcKinney et al. (2012b) revealed a correlation of ash dieback damage and the length of necrotic bark lesions, suggesting an active defence mechanism. Nevertheless, most native trees are highly susceptible and only 1% of the population is estimated to potentially produce offspring with a reduced damage probability (<10%) under the current infection pressure. Therefore a large effective population size has to be used in order to avoid genetic bottlenecks (Kjær et al. 2012, Enderle et al. 2015).

1.2. Ecology of ash

Distribution of European ash today and in history

Fraxinus excelsior is widespread in Europe. The distribution ranges from Western Europe to Russia and Iran without the most northern and southern parts up to an elevation of 1630 m in Switzerland, 1880 m in the Pyrenees and 2200 m in Iran (Pliura & Heuertz 2003). In Sweden, the distribution area of ash reaches from the southern coast up to 62° north (EUFORGEN 2009). Only very few individual trees can be found in the northern region though, most grow in the very southern part.

While forests were decreasing during Neolithic times, ash trees have been maintained in hedges and small woodlands for fodder production through pollarding and timber. During the deforestation in the 19th century, ash trees have been restrained to ravines and steep slopes forming a fragmented community. After the decrease of rural population, ash has been favoured in areas abandoned by agriculture or traditional management and dammed rivers since it spreads easily (Marigo et al. 2000). The increasing nitrogen deposition from the air stimulates the spread of ash even more (Hofmeister et al. 2004). Besides, silvicultural methods promoted ash in the last 30 – 40 years in some parts of Europe due to its high economic value (Pliura & Heuertz 2003).

Growth and traits

Ash trees reach an age of 250 – 400 years with heights from 40 – 45 m and a diameter at breast height (DBH) ranging from 160 – 180 cm (Marigo et al. 2000). They start flowering as single trees from 15 – 20 years and from 30 years onwards in forest stands. *Fraxinus excelsior* is wind pollinated, trees can be male, female or both. The winged seeds are dispersed by wind starting in autumn (Pliura & Heuertz 2003). Ash is one of the latest species to flush and one of the first to shed their leaves.

Fraxinus excelsior is found in several forest types with a scattered distribution. Fertile, pH-neutral, deep and well drained soils offer the best growing conditions (Dobrowolska et al. 2011). In addition, ash has also a high toleration against seasonal water logging and therefore favouring floodplains. Compared to beech ash has a broader range of site conditions growing on wetter and drier sites e.g. on bedrocks or slopes with scree (Marigo et al. 2000), comparable to the amplitude of oak. Besides, it is typically found on slopes or ravines mixed with maple, lime and elm. Ash is classified as an intermediate species between pioneer and climax, since it has different abilities at different ages. Ash often regenerates under a shelter of other tree species (beech e.g.), because it can sit and grow slowly for a long time in the shade. But with enough light young ash trees can grow very fast. Hence, ash regenerates highly and quickly especially in gaps, but can only compete under suitable conditions (Pliura & Heuertz 2003). The shade casted by ash is lighter than of beech letting more light to the ground and its vegetation. With a low C/N-ratio the litter is easily degradable resulting in a fast nutrient cycling, faster than most other native species. Therefore the herbal layer consists of demanding species, many invertebrates and fungi can be found in the soil and only small amounts of carbon are stored (Mitchell et al. 2014).

Regeneration

The regeneration potential of ash is very high (Pliura & Heuertz 2003). Seedlings germinate in light or heavy shade and can survive in low light for a long time. This "seedling bank" combined with a rapid growth as soon as light increases gives ash an advantage when filling gaps compared to beech or elm, which are tolerant to shade but slow growing. Those species grow in the shade of ash and form the next layer (Hofmeister et al. 2004). The density of seedlings is higher in hardwood than in mixed forests. In pure stands regeneration is often hampered by a thick layer of ground vegetation. Surveys from Belgium show a natural regeneration density up to 150,000 individuals per ha, reduced to 12,700 ind. ha^{-1} on slopes or exposed sites. A high litter layer and an open canopy reduce the regeneration density, whereas it increases along rivers (Dobrowolska et al. 2011).

Even though the temperate forests dominated by hard wood only cover 1% of Sweden's productive forest area, they are inhabited by a high number of vascular plants, fungi, bryophytes and lichens. Forests with ash often harbour a high species diversity. Especially the eutrophic elm-ash forests like the study area Dalby Söderskog which favour epigeic bryophytes because of their rapid decay of litter, relatively high soil moisture and air humidity (Diekmann 1999). Ash has a rough bark with high pH which results in diverse epiphytic communities of lichens and bryophytes, including many species of conservation concern (Ellis et al. 2012, Jönsson & Thor 2012, Lohmus & Rumel 2014, Mitchell et al. 2014). Due to ash dieback European ash is categorized as "Endangered" on the red list of Sweden since 2015, even though it used to be an important commercial timber (Lygis et al. 2014). A total stock of 6 mio m^3 reveals a minor commercial role compared to oak (30 mio m^3) or spruce (1232 mio m^3), however ash is important for some private forest owners in southern Sweden (Stener 2013).

1.3. Aim of the study

Since commercial forests with ash are facing economic losses (Pautasso et al. 2013), surveys about ash dieback have been conducted in managed forests. There is a lack of research in natural forests so far. In addition studying regeneration is important to predict development of dieback-damaged, rapidly changing ecosystems and to plan silvicultural measures to minimize the disease's impact (Lygis et al. 2014). Therefore, Dalby Söderskog forest reserve is selected as a case study to investigate the impact of ash dieback on ash regeneration. According to historical surveys *Fraxinus excelsior* was one of the least abundant tree species in 1909, but the number of trees (>10 cm DBH) increased considerably due to its high regeneration reaching a share of 40 % in 2011. In 2011 however, 56 % of the ash trees were classified as sick (Brunet et al. 2014). This brings up the question of how ash dieback influences the long term forest dynamics: Will ash disappear or can it persist as a major forest species?

Therefore, this study will investigate the regeneration of ash in 74 permanent sample plots in Dalby Söderskog. Data about abundance, height classes and damage will be collected to answer the questions: How widely affected is the regeneration by ash dieback and can patterns be detected from geographical analyses? Is the damage by ash dieback dependent on tree size? Does canopy cover of larger trees affect the dieback patterns?

2. Material and Methods

2.1. Study site

Dalby Söderskog is a small national park in southern Sweden with a size of 37 ha. It is located in the county of Skåne, 10 km east of Lund (55°41'N, 13°20'E, 50-75 m a.s.l.). The temperate sub-oceanic climate is characterised by a mean annual temperature of 7.5°C and an average annual precipitation of 650 mm. The loamy clay soil originating from the Baltic moraine during the Weichsel glacial period is calcareous and rich in nutrients with a mean pH of 6.1 (Persson et al. 1987). Hence the soil type is defined as eutric cambisol with mull humus, which is mostly wet and moist apart from the relatively well-drained river banks along a small creek flowing through the southern part (Lindquist 1938, Brunet et al. 2014, von Oheimb & Brunet 2007). A high ground water table causes several depressions to become water filled during winter. Though the draining of surrounding agricultural fields lowered the ground water table (Malmer et al. 1978).

Dalby Söderskog was used as a wood pasture and for irregular cuttings in medieval times, but is a protected reserve since 1918. According to historical sources the forest had a sparse canopy of *Quercus robur, Fagus sylvatica, Ulmus glabra* and a closed understory of *Corylus avellana* and *Crataegus* spp. from the 16^{th} to the 18^{th} century (Lindquist 1938). Periods of low grazing enabled regeneration, which form today's overstory of 200 year old oak, beech, elm, and ash. Also a few 300 year old veteran oaks remain (von Oheimb & Brunet 2007). At the end of the 19^{th} century no more grazing took place in the forest and the last significant harvests were done from 1914-16 (1,600 m^3 out of a standing stock of 8,000 m^3). From then on, secondary forest succession without active management was permitted. Only some smaller trees were cut in order to release veteran oak crowns. Since 1988 elms killed by Dutch elm disease were felled when they stood too close to hiking trails for safety reasons. Despite the former human impact, Dalby Söderskog is today considered as a near-natural forest due to its species and structural diversity: A high variety of saproxylic insects, saprophytic fungi, epiphytic lichens and bryophytes are found on the numerous large-dimensioned trees and dead wood (Brunet et al. 2014).

Several inventories were conducted in Dalby Söderskog surveying stand structure and vegetation in 1909, 1916, 1935, 1970, and 2011. Tree inventories from 1909 and 1916 reveal *Quercus robur* as the predominant species along with *Ulmus glabra* and fewer *Fraxinus excelsior*. *Fagus* used to be restricted to well-drained soils in the south eastern part (von Oheimb & Brunet 2007). The species composition changed after the protection in 1918: Oak regeneration deceased and elm spread – until the arrival of Dutch elm disease. When the numbers of elm decreased considerably, beech, ash, and, finally, oak regenerated in the gaps. Also the diameter distribution changed: Trees with a DBH of 30-

49 cm used to dominate the forest, later on the number of young and large trees increased considerably as well as basal area until 1970. Subsequently small trees (20-29 cm DBH) as well as basal area and stem number decreased again until 2011 (Brunet at al. 2014).

2.2. <u>Data collection</u>

The regeneration of ash trees was surveyed in 74 sample plots in Dalby Söderskog. The sample plots are reconstructed from the historical inventories and therefore semi-permanent sample plots. They are distributed along transect lines with a distance of 100 m between each plot and a distance of 50 m between the transect lines. The transect lines run approximately from west to east.

At each sample plot all ash trees with a minimum height of 1.3 m and up to a diameter at breast height of 9 cm were surveyed in a 16 m^2 square (4×4 m) with the permanent sample point in the centre of the square – marked by a stick. Diameter at breast height (DBH) was measured for all stems with a DBH between 1 and 9 cm. All trees smaller than 1.3 m were surveyed in a 1 m^2 square next to the centre of the plot. Hence one parcel was selected randomly with one corner as the centre of the plot (one of four parcels with dotted lines as in Figure 1). This method is in line with earlier inventories in 2010, 1969 and 1938, when trees of 1 – 8 m height were recorded in 4×4 m plots and trees below 1 m in 1×1 m plots (Brunet et al. 2014).

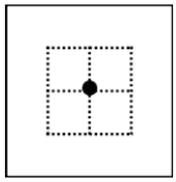


Figure 2: Plot layout. Ash regeneration <1.3 m was measured in a plot of 1x1m beneath the centre (on of the parcels surrounded by dotted lines), trees ≥1.3 m in the full 4x4m square (thick black line) with the centre of the plot in the middle.

The data collected was the plot number, an estimation of canopy cover in >2m height and the damage class of each individual ash tree. The estimation of canopy cover was done in percentage (0-100%) by eyesight. No measurement instrument was used to assess the hemispheric canopy cover. The maximum area of the plot (16 m^2) was visually checked for incoming light from above and from the sides. The canopy cover is further combined in categories: open (0-33%), half-open (34-66%), half-shade (67-83%) and shade (84-100%), see Figure 3.

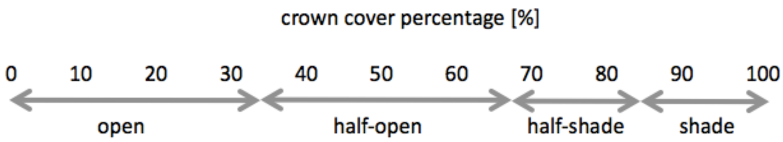


Figure 3: Canopy cover categories, divided into open (0-33%), half-open (34-66%), half-shade (67-83%), shade (84%-100%).

The damage was categorized in five classes (see Table 1). Signs of dieback are: dead shoots with coloured stains, necrotic wounds on leaves or petioles, elongated cankers on stem, wilting of leaves and branches or bark lesions free of exudates. Damage class b consists of trees without any signs of dieback but with damaged main shoot caused by browsing, shading or late frost e.g. (Figure 4). This damage class b was included in the analysis since the vitality of those trees is reduced. Hence, they are predisposed to further damage e.g. by ash dieback.

Table 1: Damage classes and description

damage class	damage description
a	healthy, unaffected by ash dieback, vital
b	main shoot damaged, possibly browsed, no (recent) signs of ash dieback found
c	slightly damaged by ash dieback, only side branches or tree seems to have recovered
d	strongly damaged and strongly affected by ash dieback, main shoot dead
e	dead

i)

ii)

iii)

iv)

Figure 4: Examples of ash regeneration in Dalby forest and their damage class (photos by Maja Dietrich, 2013); i) dead and healthy ash regeneration (damage class e and a); ii) and iii) wilting of leaves and dieback of main shoot of severely affected regeneration (damage class d); iv) browsed, but not affected by ash dieback (damage class b)

2.3. Data analysis

The program R is used for boxplots, scatterplots and statistical analysis. All statistical tests are two-sided with a 5 % level of significance, thus the null hypothesis is rejected at a p-value smaller than 0.05.

The Chi-Square-Test was used to test if the two attributes canopy cover description and damage class are independent. If the expected frequency is below 5, classes are combined. To evaluate the relevance of this coherence, Cramérs V is calculated, a coefficient of contingency for nominal variables equivalent to the phi coefficient for variables with more than 2 categories or groups. The value is between 0 and 1, the higher the stronger the correlation.

For more than 2 independent samples of non-normally distributed data the Kruskal-Wallis-Test is used to test if all samples originate from the same population (Sachs & Hedderich 1996). In the present case this test examines whether the distribution of affected trees is varying between the different canopy cover categories (H_0: Distribution of individuals with strongly affected trees are identical under all canopy cover categories. H_1: A minimum of two canopy cover categories are distributed differently.) Ties occur if values are identical and mean ranks are assigned for those tied values (Handl 2006). If ties exist between different samples like in the present case, the p-value can be influenced by them (Sachs & Hedderich 1996). Ties are already considered using the function "kruskal.test" in R and the test statistic is calculated automatically according to the modified formula. However, the result only shows whether a difference exists or not, a Wilcoxon rank-sum test finds out which categories are significantly different (also called Mann-Whitney *U* Test). From very small or very high rank sums one can conclude, that the observations originate from different distributions (Handl 2006). Hence it is tested which pairwise combination of canopy cover categories is significantly different. Because ties exist again between the samples, the Exact Wilcoxon rank sum test was used in R ("wilcox.exact") and afterwards corrected according to the Holm-Method. This is necessary due to an increased probability to reject a true null hypothesis because of multiple testing on the same data (Bärlocher 2008).

The relationship between density of trees damaged by ash dieback and canopy cover percentage was further analysed based on linear regression to provide a more detailed understanding of the relationship between these traits according to the following model(using the function “lm” in R):

$$\hat{Y} = \beta_{\square} x + \varepsilon$$

with $\beta_{\square}$ = density of damage class d per plot [stems ha-1]; x = canopy cover percentage [%]

Since the data of damaged ash densities is not normally distributed but skewed, the data was logarithmized for the log-linear regression (“log” in R).

Finally, the spatial distribution of regeneration and damage was analysed with maps made by ArcGIS in order to search for a source of ash dieback infection or check if zones with particularly many affected trees exist.

3. Results

3.1. General

A total of 739 ash trees were surveyed in June 2013. As one might expect the smaller regeneration (<1.3 m) is more abundant than trees above 1.3 m height. 505 individuals below 1.3 m were counted on all 1 m^2 plots. More than 77% of those are healthy, only 6% affected strongly by ash dieback, no individuals show only slight effects of ash dieback or are recovered (s. Figure 5). The regeneration reaching a minimum height of 1.3 m is less abundant (234 individuals on all 16 m^2 plots) and more individuals are affected by ash dieback than are not affected. Only 5% of the trees are healthy, 42% strongly damaged and 26% are already dead (s. Figure 5).

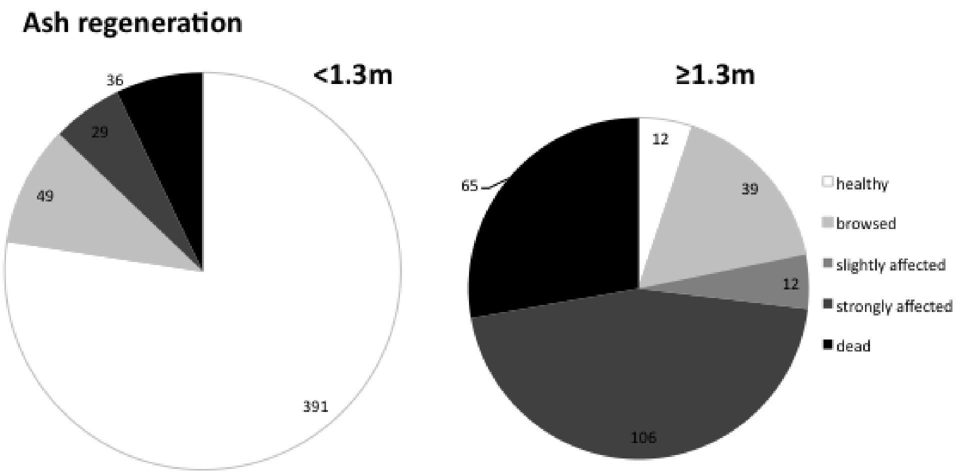


Figure 5: Damage of ash regeneration in all plots in Dalby forest

This is reflected in the mean numbers of stems per hectare (Table 2): Smaller regeneration is very abundant with a mean density of more than 64,000 stems ha^{-1} compared to less than 1,500 stems ha^{-1} higher than 1.3 m.

Table 2: Mean number of stems per ha							
damage class	a	b	c	d	e		
damage description	healthy, unaffected by ash dieback, vital	main shoot damaged, possibly browsed, no (recent) signs of ash dieback found	slightly damaged by ash dieback, only side branches/ tree seems to have recovered	severely damaged and strongly affected by ash dieback, main shoot dead	dead	Sum	Sum without dead
<1.3m [stems ha^{-1}]	53 562 (77%)	6 712(10%)	-	3 973 (6%)	4 932 (7%)	69 178	64 247
≥1.3m [stems ha^{-1}]	103 (5%)	334 (17%)	103 (5%)	908 (45%)	557 (28%)	2 003	1 447

The canopy cover conditions in Dalby Söderskog are varying. Most areas are very dense and dark. Only 7 of 74 plots in total were found in open conditions, more than half are situated in half-shade or shade (s. Figure 6). This has to be considered when analysing the regeneration according to canopy cover.

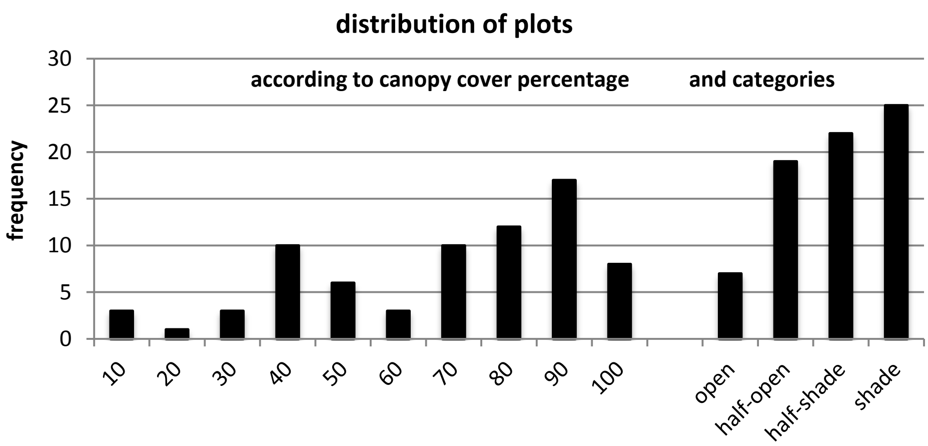


Figure 6: Distribution of plots in Dalby forest

3.2. Cartographic assessment

The Dalby forest has very dense areas in the centre, it becomes less shady towards the north and a very open patch is found in the southern end (Figure 7). However, very shady plots are found all over the forest where hardly any light reaches the regeneration underneath the canopy or shrub cover. Bigger ash trees (>10cm DBH) are found mostly in the northern part of the forest, they rarely grow in the southern very dense area. This can be explained with the high light demand of ash at a mature age. Furthermore the soil in the southern area around the stream is well drained and ash seems not able to compete against beech and other species unlike on wet and moist soils in other parts.

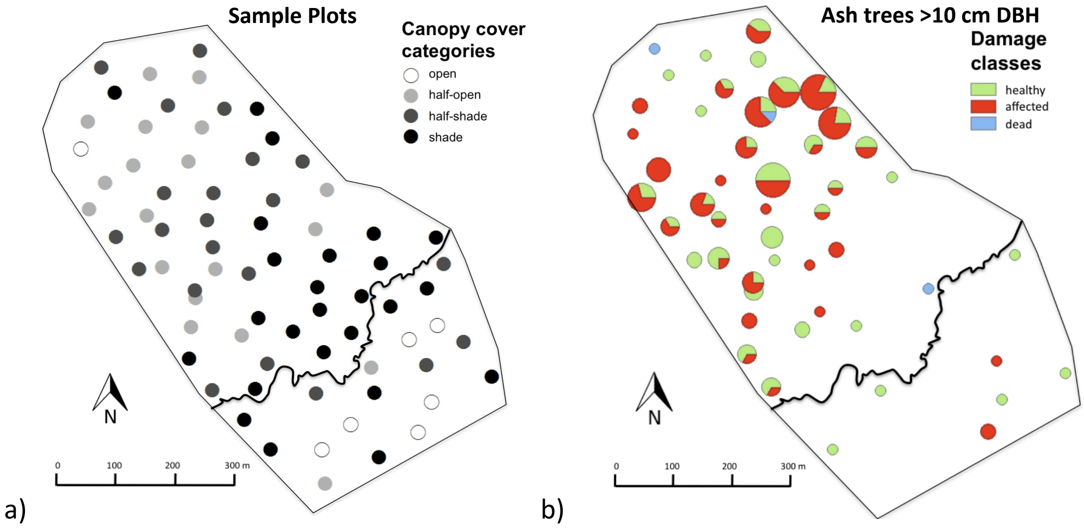


Figure 7: Localisation of a) canopy cover categories and b) trees with a DBH >10 cm (The size of the pie charts represents the number of trees per plot, a bigger chart symbolising a higher density)

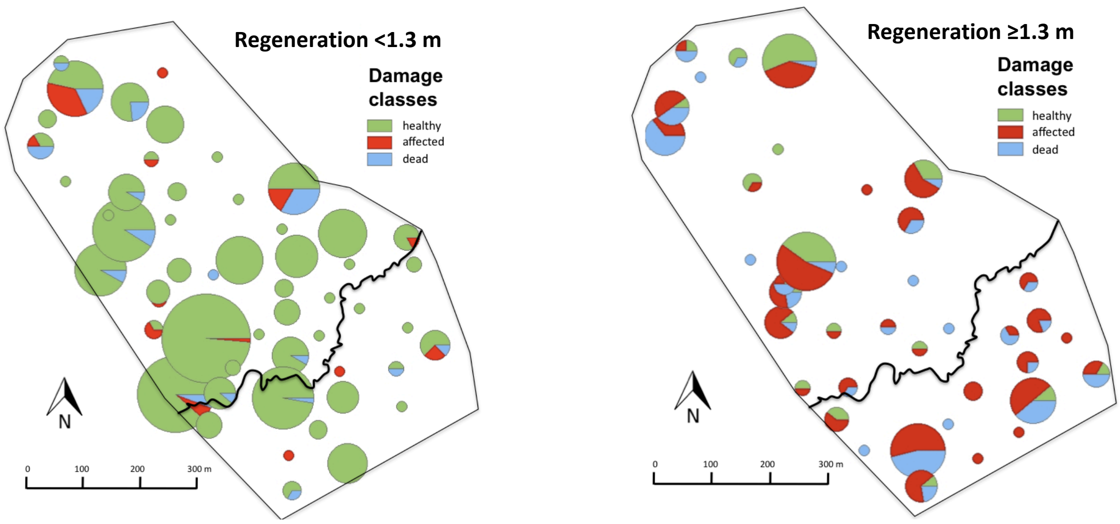


Figure 8: Localisation of regeneration a) <1.3m and b) ≥1.3m (The size of the pie charts represents the number of trees per plot, a bigger chart symbolising a higher density)

Smaller regeneration (<1.3m, see Figure 8) is found in the shady area and also spread over almost the whole forest. While bigger regeneration (≥1.3m) is sparser and found in open and half-open plots. It seems like affected small trees are located more in the north as well as healthy bigger regeneration. It is striking how in the northern region, where most of the adult ash trees are found, there is little regeneration. To sum up, no source of ash dieback infection can be detected from the maps and no zone with particularly many affected trees.

3.3. Correlation of canopy cover and damage

Figure 9 shows that the trees smaller than 1.3 m are healthy for the most part and growing in more shady conditions, whereas trees bigger than 1.3 m are mostly affected and found in more open areas.

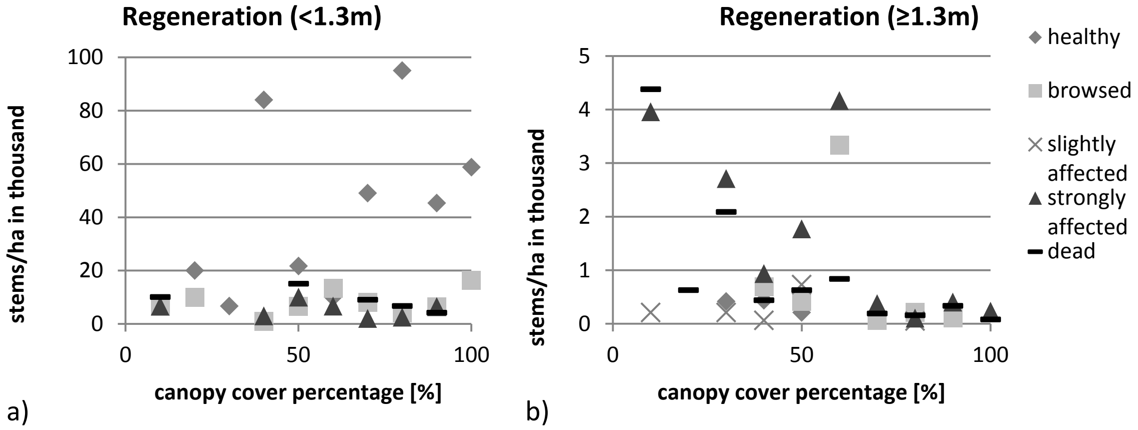


Figure 9: Regeneration density per plot according to canopy cover for regeneration a) <1.3 m and b) ≥1.3 m

Dead trees exist in both height classes and under the full range of shade conditions (Figure 10), the highest share have trees bigger than 1.3 m standing in an open area (Figure 11). Almost no clear trends of ash dieback correlating with canopy cover are visible from the diagrams. However, the share of healthy small trees seems to increase with canopy cover, whereas the share of browsed, strongly affected and dead trees decreases. Additionally, the share of strongly affected trees with a minimum height of 1.3 m seem to increase with more shade - apart from the drop in half shade conditions. These findings have to be considered with regard to the higher amount of bigger trees found in open areas as well as the many smaller trees found in shade.

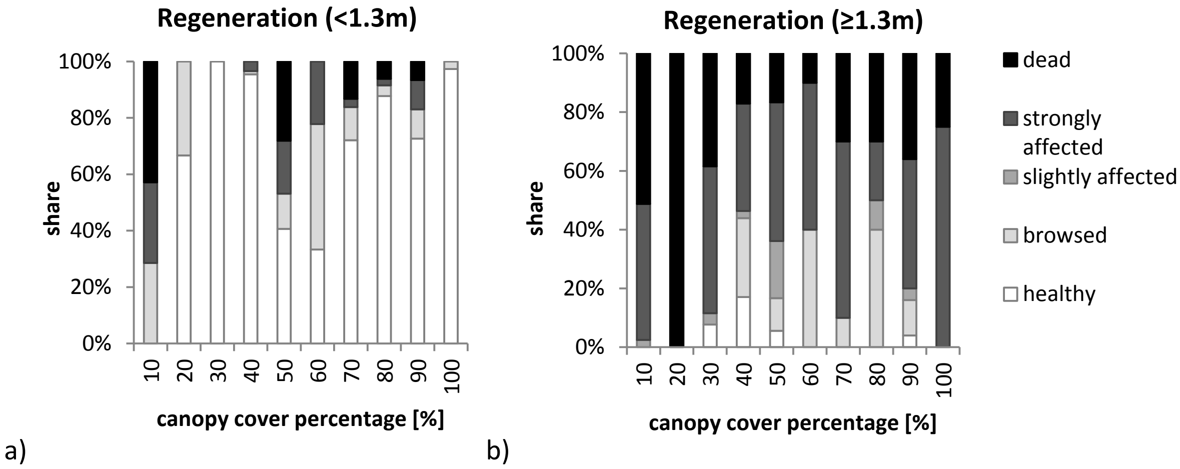


Figure 10: Share of ash regeneration in Dalby forest with different damage classes according to canopy cover percentage for a) regeneration <1.3 m and b) ≥1.3 m

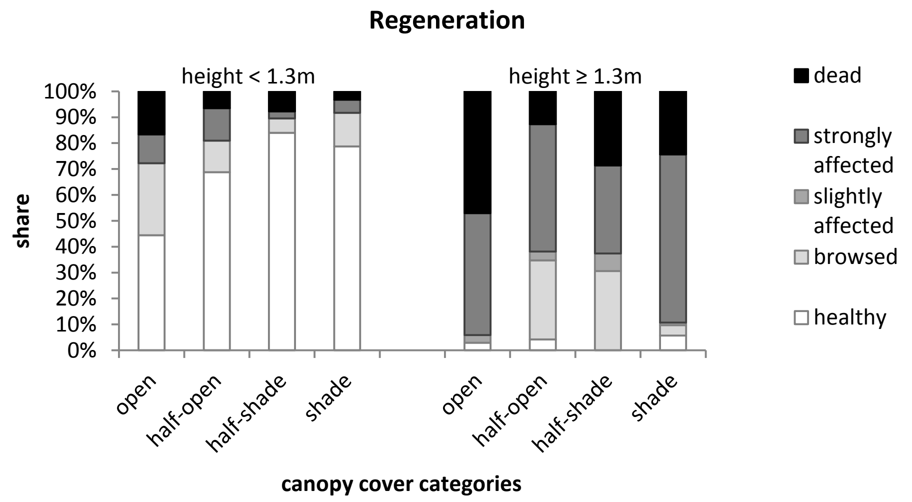


Figure 11: Share of ash regeneration in Dalby forest with different damage classes according to canopy cover categories

When comparing the regeneration data collected in 2013 with results from the inventory in 2011 the very large share of healthy trees below 1.3 m high is prominent as well as the high share of dead trees below 20 cm DBH (see Figure 12). The regeneration with a minimum height of 1.3 m seems to confirm a finding of Brunet et al. (2014): The percentage of trees classified as healthy rises with DBH. The correlation was not significant, but strong (R^2=0.58). However, regeneration smaller than 1.3 m is not in line with this correlation.

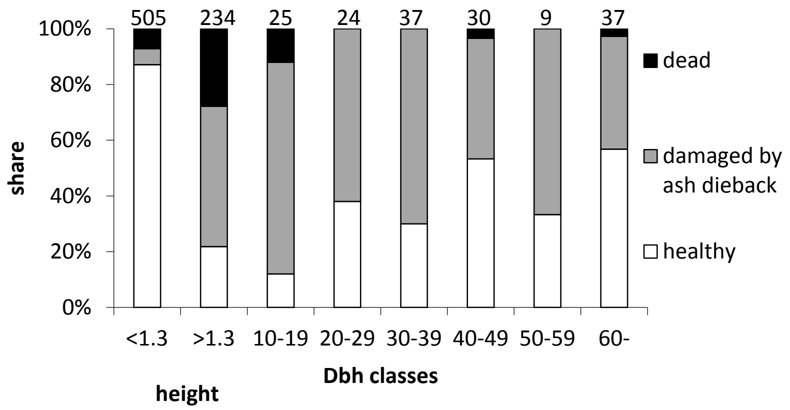


Figure 12: Share of ash with different damage classes for trees >10 cm DBH (2011) and regeneration (2013)

3.4. Contingency analysis

Due to the small number of observations for trees smaller than 1.3 m in open areas, the expected frequency for those is below 5 for damage classes b, d, and e. This is why the canopy cover categories open and half-open are merged for the Chi-Square-Test, which results in the following contingency table (Table 3). The result of the Chi-Square-Test is significant (p-value<0.05, Table 4). Therefore, the distribution of the damage classes is not independent from the canopy cover. However, the contingency coefficient is very small suggesting only a weak association.

Table 3: Contingency Table <1.3m				
canopy cover description	healthy	browsed	strongly affected	dead
open & half-open	104	12	13	12
half-shade	163	13	5	17
shade	124	24	11	7

Table 4: Chi-Square-Test <1.3m	
X^2	16.806
df	6
p-value	0.010*
Contingency coefficient	0.18
Cramers V	0.13

For trees with a minimum height of 1.3 m again some expected frequencies are below 5. That is why the damage classes a and b (healthy and browsed, not affected by ash dieback) as well as c and d (slightly and strongly damaged by ash dieback) were merged into the following Contingency Table (Table 5). The Chi-Square-Test shows a very strong significance (p-value<0.05, Table 6) and also the Cramers V coefficient is higher than above.

Table 5: Contingency Table ≥1.3m			
	healthy & browsed	slightly & strongly affected	dead
open	2	34	32
half-open	40	60	17
half-shade	5	9	6
shade	4	15	10

Table 6: Chi-Square-Test ≥1.3m	
X^2	37.426
df	6
p-value	0.000 ($1.455 \cdot 10^{-9}$)*
Contingency coefficient	0.37
Cramers V	0.28

None of the datasets are distributed normally as can be seen in the attached figures and tables (page 40). This is because regeneration is not distributed equally over the area but depending on light, seed trees and many other factors (Röhring et al. 2006). Therefore, non-parametric tests are used in the following. The more trees are found under a certain canopy cover category, the higher the variability of this dataset, because there are always empty or almost empty plots. Therefore, the range is very wide. This is true for small trees under shade (see Table 14, page 40) and for bigger trees in open areas (see Table 15, page 40), which both show the highest variance among their group.

Despite a significant Chi-Square-Test for small trees showing an association of canopy cover and damage, the Kruskal-Wallis-Test did not show any significance for any canopy cover categories. This is probably because the correlation is not strong enough, as can be seen by the Cramers V contingency coefficient of 0.13 (Table 4).

For bigger trees the Kruskal-Wallis-test shows significance for affected trees (damage class c & d) with a p-value below the level of significance 5 % (Table 7). This is also true for strongly affected trees alone (damage class d, Table 8), but not for the small number of slightly affected trees (damage class c). Therefore, different canopy cover categories imply a varying amount of trees (≥1.3 m) strongly affected by ash dieback. No other damage category was significant.

Table 7: Kruskal-Wallis-Test ≥1.3m, slightly and strongly affected	
Kruskal-Wallis-X^2	12.159
df	3
p-value	0.007*

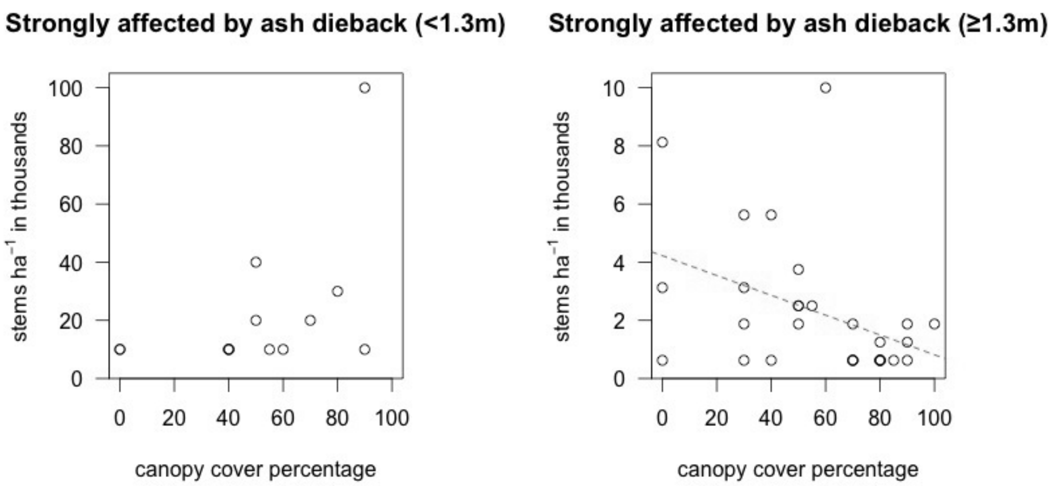
Table 8: Kruskal-Wallis-Test ≥1.3m, strongly affected	
Kruskal-Wallis-X^2	12.042
df	3
p-value	0.007*

To further specify under which canopy cover category the distribution of strongly affected trees (≥1.3 m) is different a Mann-Whitney-U-Test is calculated with the following p-values (Table 9). It shows that affected trees (≥1.3 m) are found significantly more often in open areas than in conditions of half-shade and shade.

Table 9: Mann-Whitney-U-Test ≥1.3m, strongly affected			
	open	half-open	half-shade
half-open	1.000	-	-
half-shade	**0.005***	0.299	-
shade	**0.012***	0.465	1.000

3.5. Regression

A regression was calculated to find out, how the damage by ash dieback is correlated to canopy cover percentage. The linear regression for trees smaller than 1.3 m is not significant, but for bigger trees it is (s. Figure 13, more tables and figures attached):



a) b)

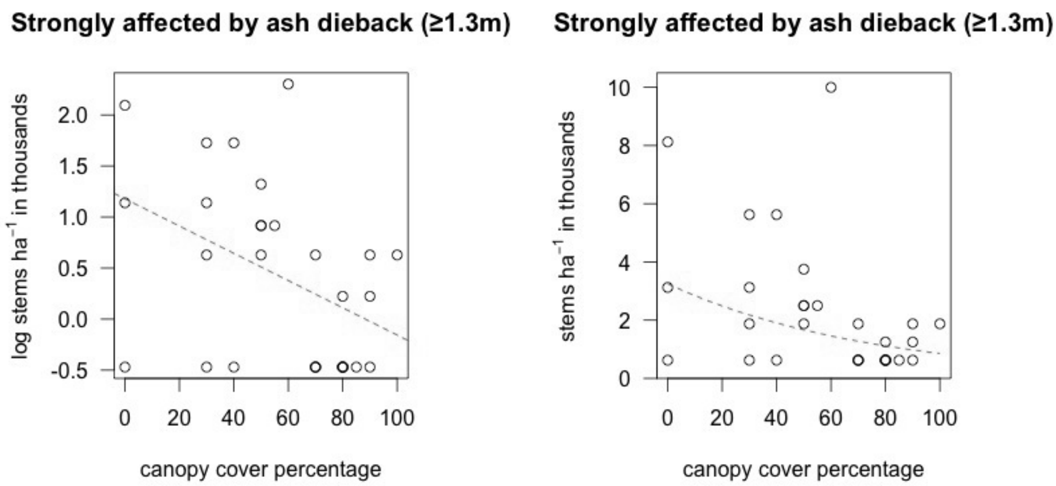
Figure 13: Scatterplot of strongly affected trees a) for regeneration <1.3m (29 trees) and b) ≥1.3m (106 trees) including regression line showing plant density in stems ha^{-1} in thousands for each plot according to canopy cover percentage

Function: $\hat{y} = 4223.28 - 34.08\ x$ with $\hat{y}$ = Stems ha^{-1} damage class d and x = canopy cover percentage

Table 10: Residuals of linear model					
Minimum	1. Quantile	Median	3. Quantile	Maximum	Standard Error
-3598.3	-1098.3	-531.5	150.9	7821.3	2189 on 27 degrees of freedom

Table 11: Statistics of linear model						
	Estimate	St. Error	t value	p-value	Multiple R^2	0.1691
Intercept	4223.3	921.6	4.583	$9.35x10^{-5}$*	Adjusted R^2	0.1383
canopy cover percentage (ccp)	-34.1	14.54	-2.344	0.027*		

The residuals are not distributed normally, as can be seen in Table 10 and the attached Figure 17 (page 41). This contradicts one of the requirements for linear regression. That is why a log-linear model is done using the logarithm of the stems per hectare. Even if the residuals are not completely regular, they become smaller and much more linear which can be seen in the QQ-Plot (Figure 17, page 41).



a) b)

Figure 14: Scatterplot a) using the logarithm of stems ha^{-1} in thousands for each plot and b) scaled back to normal values

The log-linear model (s. Figure 14) is also significant (p-value<0.05, Table 13) with a little lower p-value and a little higher adjusted R^2 (0.152) than the linear model (R^2 = 0.138). Noticeable are the residuals, which are much more symmetric and therefore imply a better fitting model (Table 12).

Function: $\hat{y}$ = 8.086 – 0.013 x with $\hat{y}$ = log (Stems ha^{-1} damage class d) and x = canopy cover percentage

With an increase in canopy cover percentage by 1 unit (%), the density of strongly affected stems ha^{-1} decreases by 1.3 %.

Table 12: Residuals of log-linear model

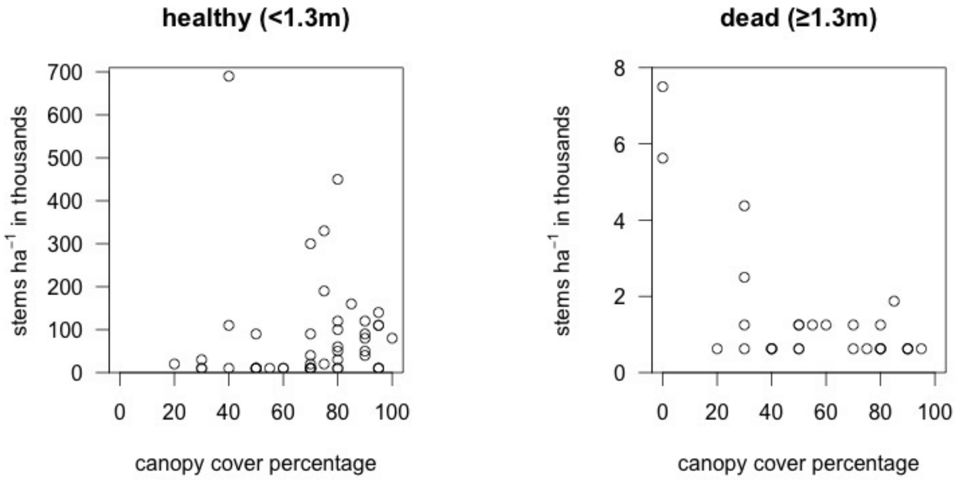
Minimum	1. Quantile	Median	3. Quantile	Maximum	Standard Error
-1.648	-0.581	0.113	0.472	1.925	0.8191 on 27 degrees of freedom

Table 13: Statistics of log-linear model

	Estimate	St. Error	t value	p-value
Intercept	8.086	0.345	23.445	$2*10^{-16}$
canopy cover percentage (ccp)	-0.013	0.005	-2.453	0.021

Multiple R^2	0.1823
Adjusted R^2	0.152

Also interesting are the following results (Figure 15): More dead (damage class e) trees (≥1.3m) were found under low canopy cover and healthy (damage class a) regeneration (<1.3m) seems increasing with higher canopy cover. However, no significance was found.



a) b)

Figure 15: Scatterplot of a) small healthy trees and b) big dead trees showing plant density for each plot according to canopy cover percentage

4. Discussion

4.1. The density and light demand of ash regeneration

The mean density of ash regeneration (65,500 stems ha^{-1}) in Dalby Söderskog is within the range of findings by other studies (12,700 – 150,000 stems ha^{-1} in Belgium, Dobrowolska et al. 2011). The reason for most of the smaller regeneration (<1.3 m) occurring in dense areas whereas bigger trees grow in open and half-open conditions can be found in the leaf structure. According to Kerr & Cahalan (2004) juvenile ash trees have sun and shade leaves, whereas adult trees only have sun leaves. The production of shade leaves enables ash regeneration to survive under a dense canopy for many years. Once ash is established, the tree can persist up to ca. 30 years in shaded conditions (Grime et al. 1988). Plus, regeneration under a closed canopy cover cannot grow as fast and become as big as sun exposed trees. Therefore the trees remain relatively small until a gap opening occurs (Emborg et al. 1998).

4.2. The health condition of ash regeneration

When combining the regeneration survey with inventory data from Brunet et al. (2014), most dead trees had a DBH below 20 cm, which is not necessarily due to ash dieback. It is also a natural process of high regeneration, competition and natural selection.

Comparing the obtained data to other studies

Surprisingly the regeneration of trees smaller than 1.3 m is mainly healthy (77%). This is probably due to taking into account the small, just lignified trees, which could be only one or two years old. Most of them might have not yet been affected, but will be in the future as soon as they grow. Comparable results were obtained from a study in Germany: 1 year-old seedlings in a nursery were significantly less affected by *Hymenoscyphus pseudoalbidus* than 2 or 3-year old shoots (Schumacher et al. 2010). This indicates that no minimum age exists for ash trees to be affected by ash dieback, but there is less damage among younger seedlings. When leaving the smallest trees aside, the regeneration (≥1.3 m) is in line with a positive correlation between tree size and health (Bengtsson et al 2014, Brunet et al. 2014, Skovsgaard et al. 2010). A higher share of healthy trees with increasing DBH means that smaller and younger trees are more affected and damaged by ash dieback. This was found in several studies (cp. McKinney et al. 2014) indicating a share of up to 95 % affected trees among the young plants. Young plants cannot recover well from infections, up to an age of 10 years they often decease 2-10 years after the first dieback symptoms. For instance, an ash plantation in Germany next to an already infected young stand was damaged by 80% already after one year. In three years 99% of the planted ash were affected and 43% dead (NW-FVA 2013). During a two year study in Sweden 17 % of all surveyed ash trees died (1-10 m) and only 6 out of 52 planted seedlings remained healthy (Bengtsson et al. 2014). Pliura (2011) found a survival rate of 10% within 8 year old trees in progeny trials and Metzler et al. (2011) discovered a mortality rate of 80% in young plantations.

The forest reserve Dalby seems to have a lower damage rate than the plantations above with only 20% of the regeneration being affected by ash dieback and 14 % classified as dead. Even when taking only regeneration ≥1.3 m into account, the share of trees affected by ash dieback is 50% and still below the reference levels from the studies mentioned before. The total regeneration in Dalby is also less affected compared to a study on natural regeneration in Lithuania by Lygis et al. (2014), which was conducted after clear-felling of dieback-affected natural forest ash stands: More than half of the young ash trees were affected by ash dieback (53.9 %), many died (16.8 %) and only about one third were visually healthy (29.3 %). The share of dead trees is comparable to this study, but for regeneration of trees with a minimum height of 1.3 m the share of dead trees is higher (28 %) in Dalby. However, it is not clear if the mortality is only caused by ash dieback or partly also by natural processes. The ash pathogen arrived in Dalby probably around 2004 (Barklund 2005) meaning the disease has attacked the forest until the current regeneration survey for already 9 years. This is a long infection period and dead regeneration could have already been decaying and decomposed. Since the data for this survey was taken in only one year, no mortality rate can be concluded. Even though it is just 5% of the regeneration (≥1.3 m) with only slight symptoms of dieback or seeming to have recovered, this finding suggests a possible partial resistance of those trees. Further monitoring is necessary for evidence.

4.3. <u>The correlation of canopy cover and damage class</u>

The damage class and canopy cover category are significantly correlated. For regeneration reaching a minimum height of 1.3 m significantly more damaged trees were found in open areas (0 – 33 % canopy cover) than in areas of half-shade or shade (66 – 100 %). And the regression model shows a decrease of damaged trees with an increase of canopy cover, even though the R^2 of 0.152 is very low. These findings are in line with a study from the Swedish island Gotland (Jönsson & Thor 2012), where traditionally managed open wooded meadows were more severely affected by ash dieback than unmanaged closed forests and semi-open grazing sites. They had a canopy closure of 51% compared to 72% in the forest and 63% on the grazing sites. Trees in open areas seem to be more easily affected by the fungal spores of *Hymenoscyphus pseudoalbidus*. However, the authors suggest that pollarding might be part of the problem, since 50% of the trees found in traditionally managed open wooded meadows have been pollarded compared to none in the forest and 18% on grazing sites. It might be possible that those trees become more susceptible because multiple infections can occur on a single tree (Bengtsson et al. 2014, Gross et al. 2014). The intense sprouting of new foliage after pollarding can therefore accelerate the dieback.

Impact of other factors

Despite the significant results, neither the correlation coefficient (Cramers V) nor the coefficient of determination (R^2) is high, likely because many factors are influencing natural regeneration: Site, tree layer composition, light, water supply, soil relief, condition of the top soil, competition by ground vegetation, browsing and distance to seed trees including their flowering frequency and seed dispersal (Röhring et al. 2006). The regression analysis was done with density values to take those factors into account, because no other current data (e.g. about soil moisture) was available. Using the share of affected trees would represent the infection rate better than total values. But compared to the counted values, the share of affected trees is depending on the total amount of regeneration and therefore varies highly. To reduce the high variability within the dataset, a stratified sample design could be more useful. Especially for an agglomerated distribution of regeneration, the stratified sample design is more accurate (Ammer et al. 2004).

And even more, mainly still unknown factors influence if a tree is affected by ash dieback: genetics (Pliura 2011, McKinney et al. 2014), vigour (Skovsgaard et al. 2010) and site characteristics other than canopy cover like wet areas and waterlogged patches (Cech 2008, Schumacher 2011). These variables could not be taken into account, but they most probably play a role in the correlation between ash dieback and canopy cover. Besides, climate conditions influence the development of ash dieback on a larger scale: The continental climate in eastern Ukraine with hot summers and cold winters may have impeded the spread of the pathogen (Davydenko et al. 2013). On the other hand, *Hymenoscyphus pseudoalbidus* is found to have a high genetic variability resulting from a variety of climate conditions throughout its life cycle (Kraj & Kowalski 2014). This helps the pathogen to tolerate different external conditions and spread easily.

4.4. Future development of ash in Dalby Söderskog

For the future development of Dalby Söderskog, an ash regeneration of 60,000 stems ha^{-1} not affected by ash dieback is present. However, it remains unclear if those will be affected in the future. Especially concerning only very few trees bigger than 1.3 m are healthy and vital (5%), even when taking the browsed stems into account the density of regeneration (≥1.3 m) is only 400 stems ha^{-1}. It seems likely, that once the small regeneration grow larger, they will become affected by ash dieback, since the regeneration comes from the same parental trees and has a similar genotype. The site conditions are not very different either. Because the forest reserve is relatively small, the fungal pathogen can spread rapidly. A decrease of ash stems in Dalby seems certain, opening the way for oak regeneration and other species (Brunet et al. 2014).

4.5. Further research

Further studies are needed to understand the consequences of ash dieback in Dalby Söderskog. A supplementary inventory with marking of individual trees can tell more about the development, the mortality rate and if regeneration trees can recover. For instance, it has been shown that the disease progresses during the winter: In a case study 42% of seedlings appeared healthy and without ash dieback symptoms in autumn but revealed to be affected in the following spring (Kirisits et al. 2012). Besides, more information about influencing factors needs to be collected to bridge the attribution gap. Site characteristics for instance, as has been surveyed in other studies: Metzler (2010) found wet sites to increase dieback, supposably because the pathogen favours a humid micro climate and has more litter to grow in when the litter decomposition is delayed. Ash regeneration itself is reduced by a high litter layer and open canopy (Dobrowolska et al. 2011). It remains open, how the correlation of dieback and canopy cover will develop in the future. The dieback might just be delayed, and should therefore be further investigated.

5. Conclusions

- case study in a national park in Southern Sweden with specific forest history, species composition, climate and site conditions, but quite typical for a natural ash occurrence since these are often found in mixed stands and along streams
- results of this study can be transferred to Denmark, Germany and other countries, because of the mild climate

- smaller regeneration (<1.3 m height) is rarely affected by ash dieback, low infection pressure
- bigger regeneration (≥1.3 m height until 9 cm DBH) is severely affected
- small regeneration likely to become affected due to similar site conditions and parental trees
- forest cover seems to influence the susceptibility to ash dieback: The damage decreases with an increase in canopy cover.
- some individuals seem to have recovered producing new shoots after dieback of side branches suggesting a partial resistance

- individual trees should be marked and monitored in a long-term to study to further analyze resistance potential
- environmental factors and competition should be included in the analysis, since tree size is depending on them

- planting of ash seedlings even less practicable since a resistance cannot be proven for young seedlings

- When searching for resistant trees and genotypes, it should be considered that ash seems less severely damaged by ash dieback in natural forests than in plantations. Whether it's due to genetics, structural diversity or a combination of several factors, natural forests are typically more robust and resilient to disturbances (Emborg et al. 2000).

6. References

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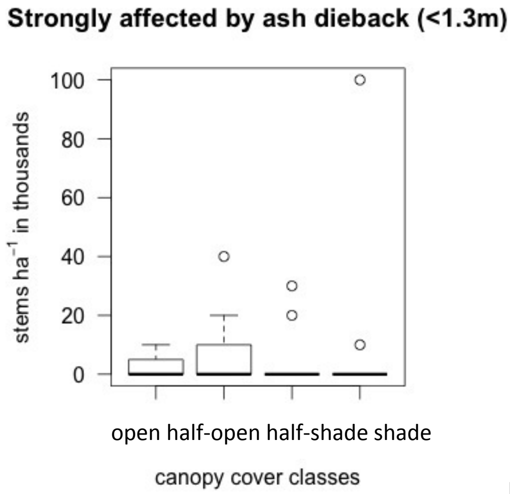
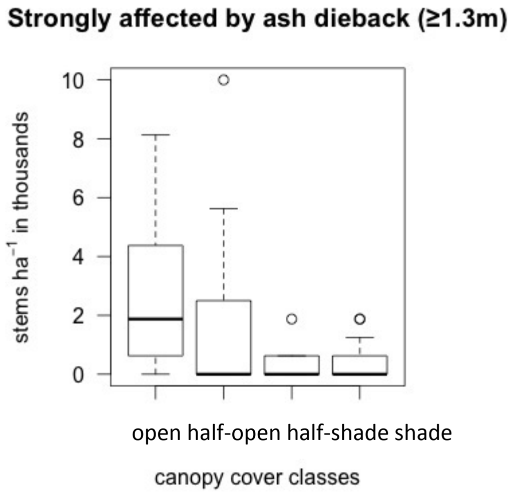
7. Appendix

7.1. Distribution of data



a)



b)

Figure 16: Boxplots of strongly affected trees a) <1.3 m and b) ≥1.3 m

Table 14: Statistics of damage class d (strongly affected) <1.3m				
	open	half-open	half-shade	shade
Min	0	0	0	0
1st Quartile	0	0	0	0
Median	0	0	0	0
Mean	2 857	5 789	2 273	4 400
3rd Quartile	5 000	10 000	0	0
Max	10 000	40 000	30 000	100 000
variance	23 809 524	103 508 772	56 493 506	400 666 667
standard deviation	4 879.5	10 173.9	7 516.2	20 016.7

Table 15: Statistics of damage class d (strongly affected) ≥1.3m				
	open	half-open	half-shade	shade
Min	0	0	0	0
1st Quartile	625	0	0	0
Median	1 875	0	0	0
Mean	2 857	1 711	227.3	350
3rd Quartile	74 375	2 500	468.8	625
Max	8 125	10 000	1 875	1 875
variance	9 095 982	6 807 383	206 304.1	360 677.1
standard deviation	3 016	2 609.1	454.2	600.6

7.2 <u>Regression</u>

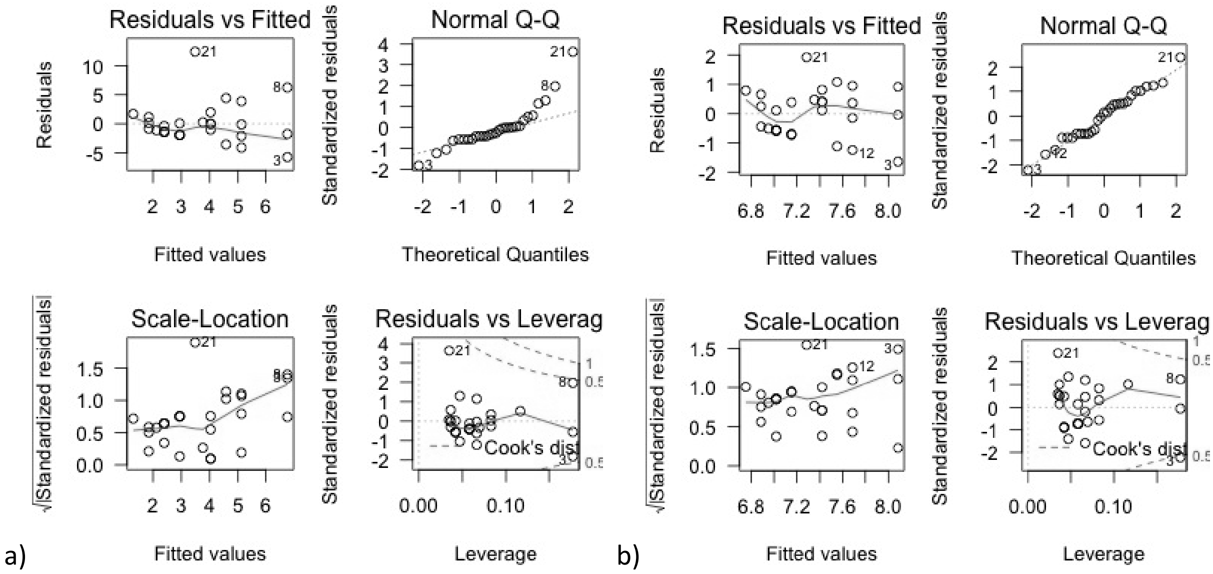


Figure 17: Statistical Analyses of Linear Regression for regeneration ≥1.3 m with a) normal values and b) logarithmized dependant variable

Regarding the statistical analyses of linear regression with normal values (Figure 17a): A pattern in the plot “Residuals vs Fitted” shows that the variance increases with higher fitted values and the curve suggests a local bias contradicting the assumption of a linear relationship. Besides, the quantile-quantile (Q-Q) plot doesn’t show linearity of the residuals. The curve in plot “Scale-Location” implies no constant variance again and data points beyond the Cook’s distance (red line 1) suggest that the sample doesn’t represent the population well. All these factors contradict with the requirements for linear regression. Comparably the residuals of the log-linear regression are more linear and therefore better fitting (Figure 17b).

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