Genetics of ash dieback
Gösta Eriksson and David Clapham

Department of Plant Biology SLU

Preface

The objective of this report is to summarize the genetic knowledge as regards the serious ash shoot dieback disease. Since the turn of the century many geneticists have studied various genetic aspects of this disease, which called for a review of the current knowledge of genetic variation in disease resistance. Our aim is to highlight important results from the various investigations. We begin with general remarks on genetics and disease resistance and then present genetic variation in ash shoot dieback within and among populations. We briefly present variation among ash species. Finally, we discuss breeding and genetic conservation of *Fraxinus excelsior*.

Cover photo by Barbro Ekberg

Genetics of ash dieback

1.   Distribution of ash and ash dieback

Common or European ash (*Fraxinus excelsior* L.) is a forest tree regarded as wild over the greater part of Europe and the Caucasus and in the southern half of Sweden. It is wind-pollinated, and wind spreads the seed. Some individuals have only male flowers, some only female flowers, and some have both. Its timber is highly regarded for e.g. axe shafts, tool handles and some kinds of furniture. The problem with cultivating ash is that to grow well it requires a very moist soil rich in humus and nutrients, but will not tolerate stagnant water. It can seldom be grown over a large area (e.g. Vedel and Lange, 1965). This makes it mostly unattractive for modern commercial forestry. Ash grows in gardens, and until recently was planted communally in towns. It accounts for 0.1% of the trees in Sweden (Nilsson et al., 2019), is a host for many animals and plants, and has great significance for biological diversity.

In 1992 a number of dead ash trees were discovered in Poland, and the disease was designated 'ash dieback.' Symptoms of the disease (see e.g. McKinney et al., 2014) include dieback of shoots, twigs and branches in the crown and necrotic lesions on leaves and in the cambium. Branch dieback leads to growth of shoots from dormant buds and the tree takes on a bushy appearance. Infection by the fungus *Hyoscyamus fraxineus* was associated with the disease (Przybyl, 2002; Timmerman et al., 2011) which soon spread across the border to Lithuania and then to most of the western and central European countries, where it is a serious problem. The rapid spread of the disease in Lithuania, and its seriousness, is shown in Fig. 1; the incidence of symptom-free trees fell from 35% in 2002 to 8% in 2008. This rapid spread has led forest geneticists to study if there is genetic variation in resistance to ash dieback. Here we can begin with some relevant general genetic questions.
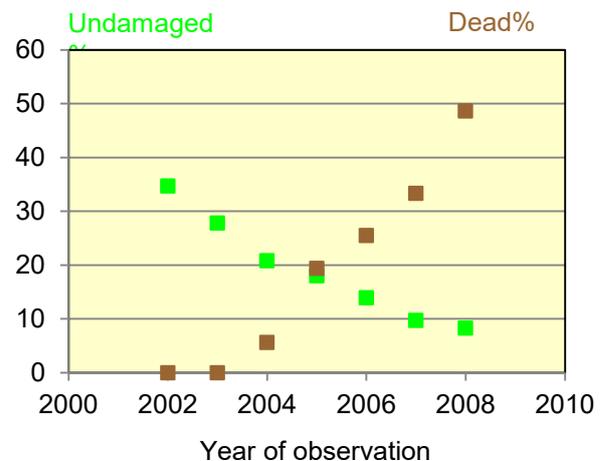

Figure 1. Development of ash dieback over the period 2002–2008 in a permanent trial area with ash in Lithuania. Whereas in 2002 there were no dead trees, by 2008 almost 50% were dead, and fewer than 10% of the trees had no external sign of dieback (after Pliura et al., 2017).

1.1 Genetics and resistance

How can genetics contribute to the conservation of the common ash in Sweden and elsewhere? A first question is: are there resistant trees in existing natural populations? Are there resistant populations in neighboring countries that can be exploited as seed or as cuttings for planting in infected areas? Or trees from the resistant populations that can be crossed with native trees to determine if resistance is qualitative—determined by genes at one or two chromosome loci—or quantitative, i.e. polygenic—determined by genes of small effect at many loci.

If the inheritance is monogenic, it is practicable to build up a resistant population by crossing selected superior genotypes performing well in the appropriate Swedish environment of interest with the resistant trees and selecting resistant progeny; or in principle the gene for resistance can be sequenced, cloned and transferred to susceptible genotypes by genetic transformation; or resistant genotypes can be identified by partial genetic sequencing.

One can speculate if qualitative inheritance is likely in a long-lived tree such as ash. A pathogenic insect or fungus has many generations during which various mutations can arise that break down the resistance of the tree, so one would expect resistance to be polygenic. The review of genetic studies by a Danish group (McKinney et al. 2014) strongly supports the polygenic basis of resistance. Traditional tree breeding programs are designed to enable selection for favorable traits under polygenic control with moderate heritability (see e.g. discussion in Eriksson et al., 2020). Nowadays molecular biotechnology entices with possibilities for (a) mass sequencing to identify DNA sequences that affect economic characters and (b) genetic transformation, and more recently genetic editing, to construct trees with appropriate genetic sequences from several different relevant genes. For ash these techniques are still at an early stage of development, even if a genome sequence has been published for European ash (Sollars et al., 2017) and Hebda et al. (2021) have obtained stable transgenic callus lines.

The effects of pathogens vary from very slight to the death of the host. The following is a useful classification originally suggested by Woodcock et al. (2019):
1. Complete resistance: essentially no damage to the host
2. Partial resistance: limited effects of the attack
3. Tolerance: symptoms develop and growth is affected negatively but the host does not die.

If complete resistance cannot be obtained, tolerance can be an acceptable alternative.

Various types of response, or phases in the response, to a pathogenic attack have been identified (cf Santos et al. 2017), such as:
1. Morphological changes lead to the pathogen being avoided
2. The host recognizes the pathogen
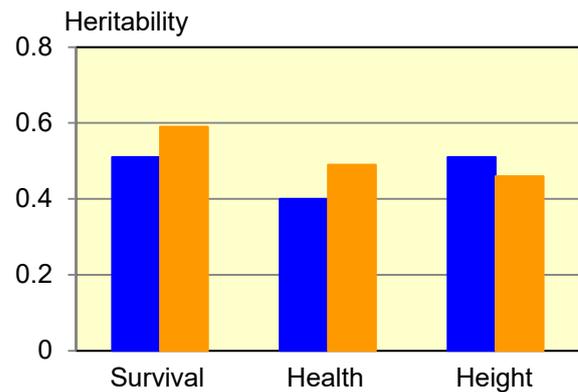3. Resistance genes are activated in the host

Figure 2. The heritability for the characters survival, general health and height in two Lithuanian field trials with 320 progenies from open-pollinated trees from 21 populations. Evaluation at age 8 years (after Pliura et al., 2011).

False resistance can also occur, without the host being exposed to the pathogen. The pathogen may attack the host only at a specific stage of the host's development. Or one can cite the false resistance of the elm *Ulmus glabra* ssp. *glabra* to Dutch elm disease in the northernmost area of its distribution in Sweden. The insect carrying the pathogenic fungus *Ophiostoma novo-ulmi* that causes the disease elsewhere in the elm's distribution does not exist north of *Limes norrlandicus* in Sweden. Such apparent resistance can deceive the tree breeder.

It is well-known among geneticists that accurate estimates of narrow-sense heritabilities can only be attained in well-designed trials. Clonal trials consist either of cuttings or grafts from individual trees. Clonal seed orchards can also be used for estimation of clonal repeatabilities (broad-sense heritabilities) since each clone is usually represented by a large number of grafts. For both types of estimate it is important that the trials/orchards or plantations have randomly planted replicates. Seed orchards normally meet these demands and can therefore be used to estimate clonal repeatabilities.

Figure 3. Variation in the occurrence of totally damaged trees from grafts from 39 Danish ash clones studied in a Danish seed orchard. Clones 30 and 37 proved to be the same clone. Clones were selected during the period 1934–1997 before ash dieback had reached Denmark (after McKinney et al., 2011).
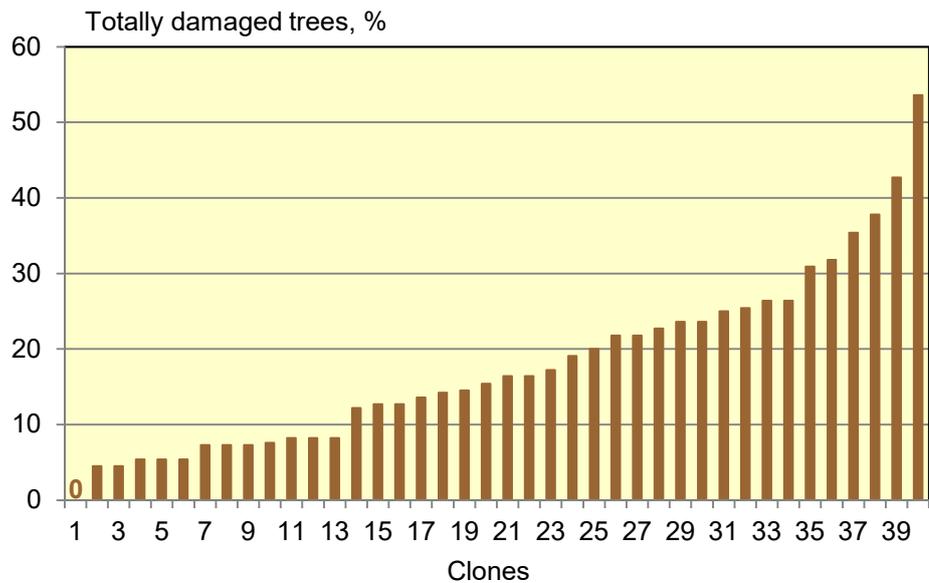
2. Genetic variation within populations

Two provenance trials (Pliura et al., 2011) in Lithuania with 21 populations from a row of countries consisted of progenies after open pollination. Heritabilities could be calculated for the traits of interest. As shown in Fig. 2, the heritabilities were high (0.40–0.60) for the three traits survival, health and tree height. Since the calculations did not take into account that the families were derived from 21 different populations the values are probably somewhat exaggerated. Even so, the results are encouraging and indicate good possibilities for progress through selection.

In Denmark over the period 1934–1997 a large number of ash trees with good stem quality were chosen for establishment of two clonal seed orchards; one on Zealand and one on southeastern Jutland (McKinney et al, 2011). The trees were selected before ash shoot dieback reached Denmark. Grafting was carried out in 1998. An inventory of ash dieback on the grafts was made during 2007–2009. The results from the latest estimation of damage in one of the orchards is shown in Fig. 3. The variation among clones is very great; everything from absence of dead grafts to more than 50% dead. The extent of damage was strongly correlated between the two clonal seed orchards and between different years in the same orchard. Each clone was

represented by 50 grafts randomly placed in the orchard. This satisfied the requirements for estimation of clonal repeatability. Fig. 4 shows that with one exception H^2 varied between 0.39 and 0.54. Thus, a considerable part of the observed variation in ash dieback is genetically determined. Possibilities to select trees that are resistant to dieback are therefore good, even if the frequency of resistant trees is very low. The clones studied came from diverse parts of Denmark. Nothing suggested the place of origin had significance for the frequency of damage. It was observed that trees that had late growth cessation were more often damaged than trees with a short growth period. It is therefore possible that the resistance is due to the apparently
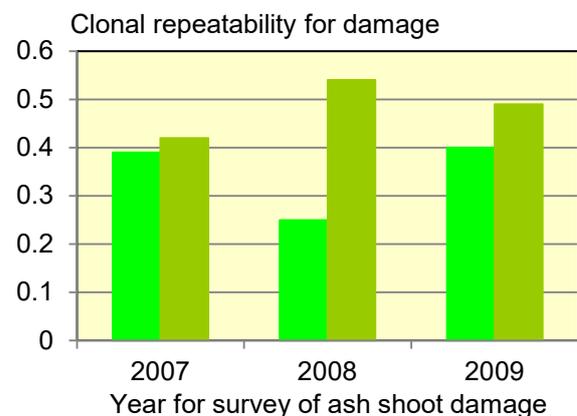

Figure 4. Clonal repeatability (i.e. broad-sense heritablility) for damage from ash dieback. The two colours represent the two clonal orchards investigated (after McKinney et al., 2011).
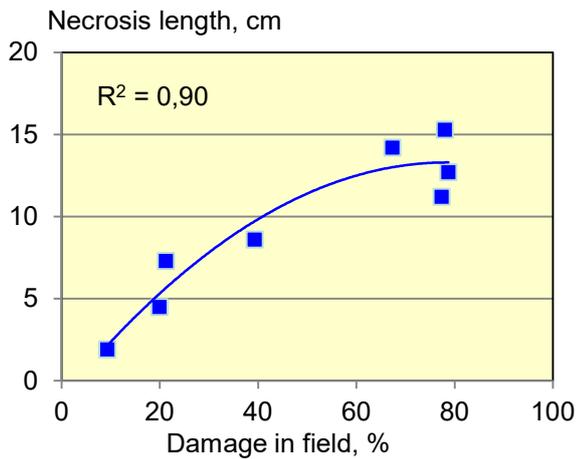
5

Figure 5. The relationship between damage level in field and length of necrosis in offspring from eight Fraxinus excelsior *clones artificially inoculated with* Hymenoscyphus fraxineus *in a Danish investigation. After Kjær et al., 2017.*
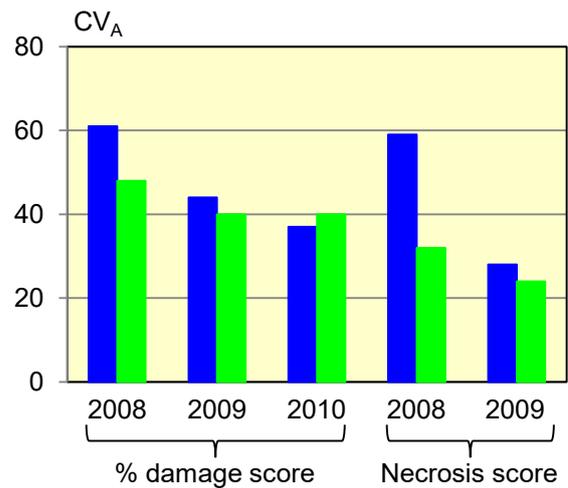

Figure 6. Coefficient of additive genetic variance for percent damage score and necrosis score recorded years 2008 and 2010 in two field trials (blue and green) with 99 and 101 open-pollinated families established in western Denmark in 2004. After Kjær et al., 2012.

resistant trees not being exposed to the disease-causing fungus; a case of false resistance. Since the least damaged trees over the three years had little damage whereas the most attacked trees showed increased amounts of damage from 2007 to 2009, the indication is that the "resistant" trees had an inborn capacity to reduce the attack from *H. fraxineus*. It can be an example of activation of resistance genes.

In the Jutland orchard the genetic variation in tree height was great whereas in the other orchard it was rather limited. In both orchards tree height was weakly negatively correlated with frequency of damage. The biggest trees had fewer damages than the least grown trees.

Progenies from a representative selection of 8 clones from the 39 Danish clones in the above study were inoculated with *H. fraxineus* to compare ash dieback in the field with that in the progenies (Kjær et al., 2017). Fig. 5 shows that occurrence of damage in the field is strongly correlated with necroses in the progenies; the R^2-value was 0.90. The inoculation experiment further indicated that the narrow-sense heritability for resistance to ash dieback varied between 0.37 and 0.53 in the three field trials with the inoculated material. These are high values

that favour attempts to increase the resistance to ash dieback. Another interesting observation from this investigation: the clone that was most resistant showed only small patches of necrosis after inoculation. It appears that this clone can protect itself from a spread of the fungal attack; in other words that the host tree can respond actively to the fungal attack.

Two field trials with 99 and 101 open-pollinated progenies from 14 populations in western Denmark were established in 2004 (Kjær et al. 2012). The selection of the mother trees took place before the arrival of *Hyoscyamus fraxineus* in Denmark. Each mother tree was represented by 32 seedlings in each trial with 4-tree plots. Percentage damage score (PDS) in five classes and necrosis in four classes were recorded during three consecutive years, 2008–2010. Ten progeny trees from seven OP-families were genotyped by three microsatellite markers to test if the OP-progenies could be classified as half-sib families, which is of importance for reliable estimates of genetic parameters. This marker study suggested strongly that the OP-families were half-sibs.
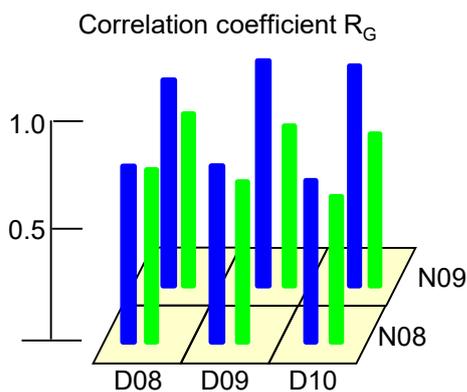
Figure 7. The genetic correlation coefficient between percent damage score (D) and necrosis score (N) recorded years 2008–2010 in two Danish field trials (blue and green) with 99 and 101 open-pollinated families. The field trials were established in 2004. After Kjær et al., 2012.

The difference among populations with respect to damage was non-significant with Q_{ST} estimates close to zero. This must be attributed to limited distances among the populations in this wind-pollinated species; with a maximum distance a little less than 150 km.

No Op-family was symptom-free and the breeding values that were calculated showed that only one mother tree is expected to give a progeny with fewer than 10% with symptoms. An additional 4 mother trees are expected to give a fairly healthy progeny, <20% damage. High coefficients of additive genetic variance, 37–61%, were noted for percent damage score all three years of observation while the corresponding estimates for necrosis score were slightly lower, 24–59% (Fig. 6), which indicated substantial genetic variation for these two traits. Moreover, the genetic correlation coefficients for PDS between trials and years were almost identical, indicating that the interactions genotype x trial and genotype x year were insignificant, which simplifies breeding. However, the site conditions of the two trials might not have varied much. The genetic correlations between percent damage score and necrosis were strong, especially in the "blue trial" (Fig. 7).
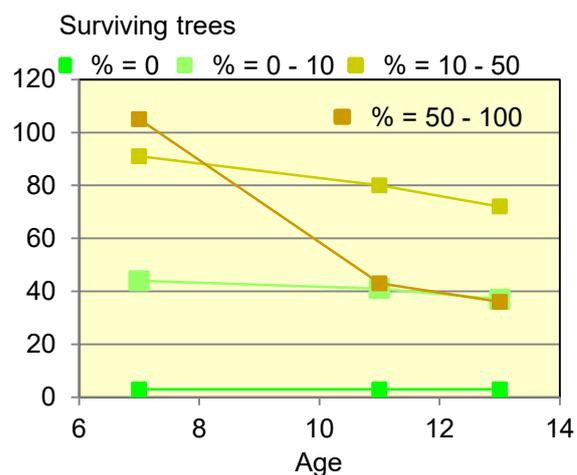

Figure 8. The number of surviving trees at ages 7, 11 and 13 years according to class of percentage damage in trees with ash dieback (after Lobo et al., 2014).

Unless the parent population is so small that inbreeding becomes a problem in coming generations, it was pointed out that the high level of additive variance combined with limited genotype x environment interaction constitute a good platform for progress in the improvement of ash health since it is expected that progenies from the healthy trees will dominate in seed dispersal in the coming generation.

Another Danish study (Lobo et al. 2014), aiming at a quantitative estimate of ash dieback, was based on 43 open pollinated families from two sites on Zealand and two on Jutland. The mother trees were chosen before ash dieback had reached Denmark. The progenies were planted in two trials, where damage and growth were registered up to an age of 13 years in the first (trial A) and to 7 years in the second (trial B). Trial B lacked fencing and faced severe competition from other plant life. Crown damage was assessed in five classes according to the percentage of damaged trees.

Survival at age 13 years in trial A was 31% with big differences among families, but no family was completely damage-free. There were similar big differences in trial B, where the average survival at age 7 was 48%. Fig. 8 shows the development of mortality from 7 to 13 years of age for four classes of damage. Most positive
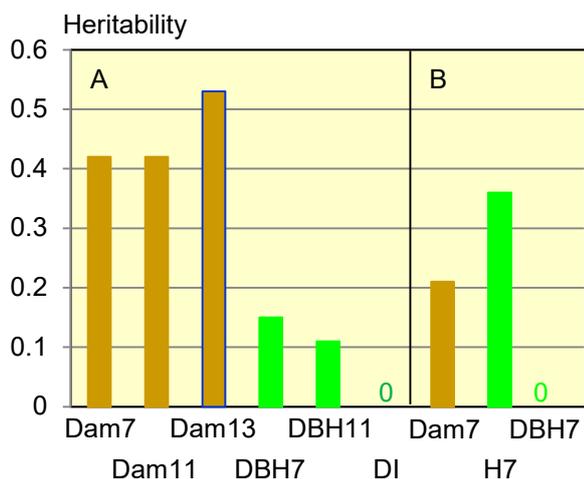
Figure 9. The heritability for growth and damage from ash dieback in two experiments, A and B, with progenies after open pollination from 43 ashes in four Danish stands. Mother trees were selected before ash dieback reached Denmark. Dam = % damage at age 7 or 11, DBH diameter at breast height at age 7 or 11, DI = increase in DBH from age 7 to 11 years (after Lobo et al., 2015).

Figure 10. Average length of necrosis 14 months after inoculation of offspring from five ash clones (after Lobo et al., 2015).

is that the three trees that were symptom-free at 7 were still symptom-free at age 13. In sharp contrast, there survived only 36 of the original 105 trees placed in the category 50–100% with damage. Variation in susceptibility to ash dieback varied greatly.

Proof for this is the high heritabilities for percent damage in trial A (Fig. 9; Lobo et al. 2015). The heritability for damage was considerably lower in trial B. Competition from other plants and the influence of animals probably explain the lower heritability estimates in trial B.

The correlations between estimates of damage at 8, 11 and 13 years were very high, varying from 0.88 to 0.97. This indicates that registration of damage one year gives reliable information about percentage damage another year. This considerably facilitates breeding since choice of resistant individuals can occur at a low age, which increases the profitability of breeding per unit time. As expected, there was a negative correlation between percentage damage in different years and diameter at breast height at age 12, -0.69 – -088. Trees with severe damage grew worse than trees with minor damage.
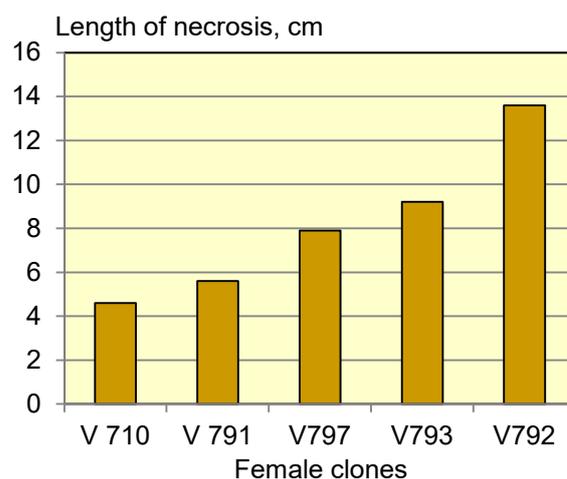
The correlation between percentage damage in the two trials was moderately strong, 0.64. A possible reason why the correlation wasn't stronger was the environment of trial B, which prevented the 'genetics' from fully emerging. A conclusion from the results of this investigation is that breeding for resistance to ash dieback is wholly feasible.

To establish the connection between mother tree and progeny regarding ash dieback, the progenies of clones with known resistance to *H. fraxineus* were inoculated with pathogen in a Danish progeny trial (Lobo et al., 2015). A strain from Norway was used for inoculation. Of the 123 trees in the progeny the father was established for 46 trees with the help of four DNA markers. These trees belong to groups of full-sibs while the other 77 trees were pollinated by trees outside the plantation. Besides length of necrosis the percentage of damage to the crowns was estimated. For both characters, strongly significant differences were found. This is illustrated for length of necrosis in Fig. 10. The correlation between parent and progeny was considerably weaker for both characters when it was based on full-sib families than on half-sib families (Fig. 11). The number of trees was very small in certain full-sibling families, so that the precision of damage estimation was low for these families. The strong correlations for the open-pollinated
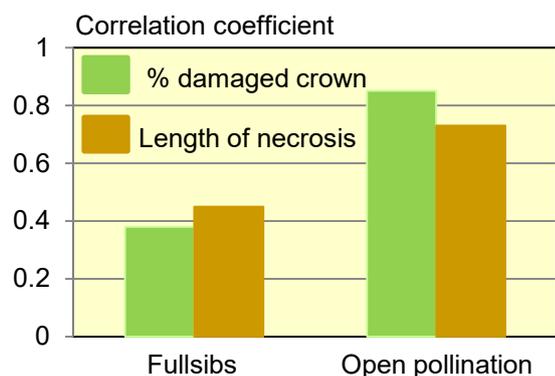
Figure 11. Correlations between parents and offspring as regards percentage crown damage and necrosis length after inoculation with a strain of H. fraxineus in a Danish progeny trial (after Lobo et al., 2015).

material indicated that inoculation can be a successful path in resistance breeding.

At ages of 15–25 years damages such as leaffall (= ash dieback) were repeatedly registered in two southern Swedish clonal seed orchards of ash (Stener 2013 and 2018).The 100 clones in the first orchard and 104 in the second were chosen before ash dieback had taken over in Sweden. In addition the form of miscoloring of boughs and their respective branches were registered. Average death rate was 7–8% in both seed orchards. On the other hand, diameter at
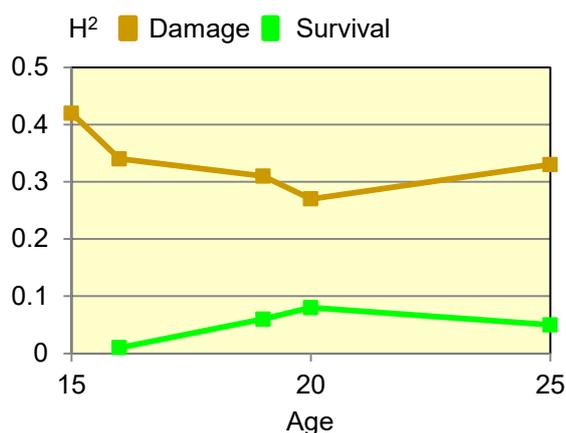

Figure 12. Development over time and clonal repeatability (broad-sense heritability) for survival and damage to stem and older branches in clones in two southern Swedish ash seed orchards with 100 and 104 clones respectively. Survival and damage were estimated according to 10 classes (after Stener 2018).
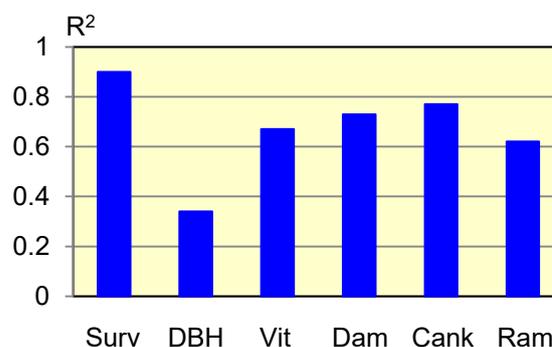

Figure 13. Explanatory power (= genotypic correlation) for the relations between the same characters in two southern Swedish clonal ash orchards at age 25. Surv = survival, DBH = diameter at breast height, Vit= vitality, Dam = damage, Cank = stem canker, Ram = ramicorn branches (after Stener 2018).

breast height differed considerably. In the first orchard (here designated Sn) the mean value for diameter at breast height was 104 mm at an age of 25 years while in the other orchard (designated Tr) it was 171 mm at 22 years. In Fig. 12 are shown the clonal repeatabilities, H^2, for plants in the Sn orchard that had the most recordings but worst growth. A hint that H^2 for damages diminishes with time is apparent. A reason can be that the number of trees that were registered drops with time owing to reduced survival. Furthermore the damages that occur over the various years can be strongly influenced by the particular weather conditions at the various timepoints. Of interest were the strong correlations between the damages in each year in the Sn orchard, with the degree of explanation varying between 80 and 97%. The correlations were weaker in the Tr orchard, 64–84%. Perhaps the greater death-rate in the Sn orchard can explain the differences, since the genetic estimations of the damages are more certain owing to the greater number of grafts tested.

Since many of the clones were common to the two seed orchards it was possible to calculate the genetic correlations between characters in the two orchards. Except for breast height diameter the correlations (see Fig. 13) are strong for all the characters, explaining over 60% of the variation. This indicates that interaction be-
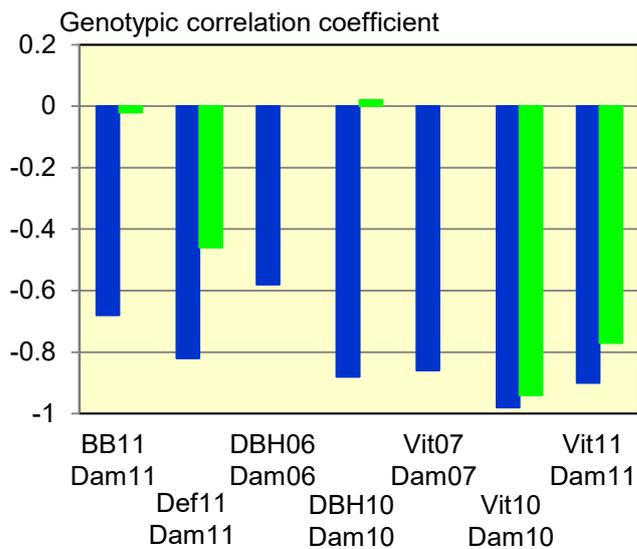
Figure 14. The genotypic correlations between various traits a certain year (11 = 2011, 06 = 2006, etc.) in two Swedish seed orchards (blue and green) with Fraxinus excelsior. *Assessments in the green orchard were carried out only in years 2010 and 2011. BB = bud burst, Def = defoliation, DBH = diameter at breast height, Vit = vitality, Dam = mean for three types of damage: damage of leader, damage of older shoots and stem damage (after* Stener 2013)

tween clone and test location is of little significance. Furthermore, the evaluation of ash dieback can be carried out at one location. A reservation, however, is that the two orchards are in the same climate region in Skåne, and both on agricultural land. This implies that the interaction clone x location is somewhat uncertain and may not apply in general. A strong genetic correlation between diameter at breast height and damage was noted for the Sn orchard at age 10 (Fig. 14, blue), explaining 88% of the variation. In contrast the correlation was absent in the Tr orchard (green DBH10 – DAM10). The genetic correlations between diameter at breast height and vitality on the one hand, and the three types of damage on the other, were very strong in the Sn orchard, explaining 81–100% of the variation. The correlations in the Tr orchard give a less clear-cut picture, varying from 5% positive to 100% negative. As mentioned above, the calculated genetical correlations were less precise in the Tr orchard, which can have contributed to the differences in the estimates between the two seed orchards.

Budburst in the spring and leaf-fall in autumn were studied to see if these phe-

nological characters were correlated with various kinds of damage. In the Sn orchard, budburst and defoliation were strongly negatively correlated with damage to stems (Fig. 14). In contrast there was no correlation for budburst in the Tr orchard. The increasing sensitivity with late growth cessation seems to be general, with the strong correlation between diameter at breast height and vitality for the Sn orchard explaining 88% of the variation. As noted above, the precision of estimating genetic correlations is lower in the Tr orchard. The strong correlations in the Sn orchard between various types of damage and vitality are encouraging for breeding since one can obtain satisfying information about resistance by registering one type of damage. In the choice between laying down much time in describing the overall kinds of damage in individual trees and analyzing several trees it is more advantageous to focus analysis on more trees.

In a study of ash dieback in 4 clonal seed orchards in western Germany (Enderle et al. 2014), the number of clones in the orchards varied from 50 to 76. As in many other cases the clones had been chosen before the ash dieback had arrived. Growth and stem quality were the selection crite-
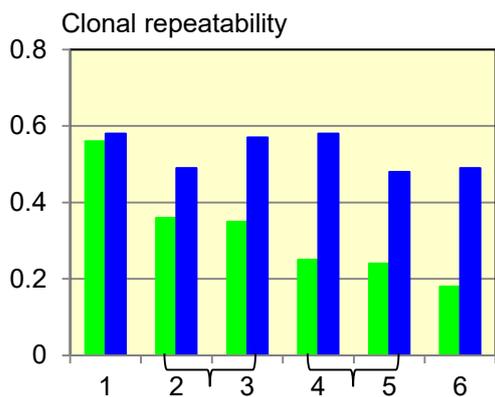
Figure 15. Clonal repeatability (broad-sense heritability) for leaf fall (green) and for epicormic shoots (blue) in four German clonal seed orchards of ash with 50–76 clones in each orchard. 2 and 3, as also 4 and 5, are the results from the same orchard with observations over 2 years (after Enderle et al., 2014).
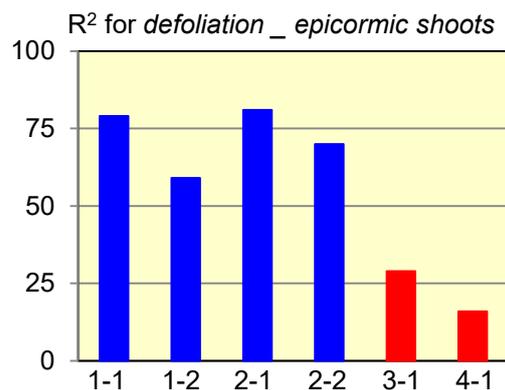

Figure 16. Explanatory power (genotypic correlation) in percent for the correlation between leaf fall and frequency of epicormic shoots in four clonal seed orchards of ash. The four columns on the left are built on two years' observations in two orchards in Rheinland-Pfalz and two orchards in Baden-Wurttemberg (red columns). The first figure indicates orchard and the second figure the year of observation (after Enderle et al., 2014).

ria.

In the two orchards in Rheinland-Pfalz the damages were fewer than in the two orchards in Baden-Württemberg, where wholly undamaged trees were absent. In the orchards in Rheinland-Pfalz, the number of undamaged trees decreased drastically from 2012 to 2013. Clonal repeatability (H^2) was calculated for leaf-fall and for formation of epicormic shoots in these ash orchards. Epicormic shoots occur in trees that are exposed to various kinds of stress such as with ash dieback and subsequent leaf-fall. The researchers argued that formation of epicormic shoots is a better measure of the effects of ash dieback than leaf-fall. H^2 is presented for epicormic shoots and for leaf-fall in Fig. 15. In two of the orchards observations were registered in two successive years. The values for H^2 were continuously high with respect to epicormic shoots, 0.40–0.60. In contrast H^2 for leaf-fall varied considerably, 0.18–0.56. Of interest was that estimations of H^2 for leaf fall were nearly identical in the two orchards when they were registered in two successive years: 0.24 and 0.25 for the one orchard and 0.35 and 0.36 for the other. One of the aims of this study was to investigate the relationship between frequency of epicormic shoots and leaf-fall. Six statistically significant correlations were noted between these two characters (for four seed orchards and the two years of observations in two of the orchards). Sometimes it is easy to demonstrate significant correlations if one analyses a large number of clones. Of greater value is to estimate how much of the variation is explained as in Fig. 16. Here we see that the correlation between the two characters does not exceed 30% in either of the two orchards in Baden-Württemberg (red columns) despite statistical significance. The correlations are much stronger in the two orchards in Rheinland-Pfalz, all explaining more than 50% of the variation.
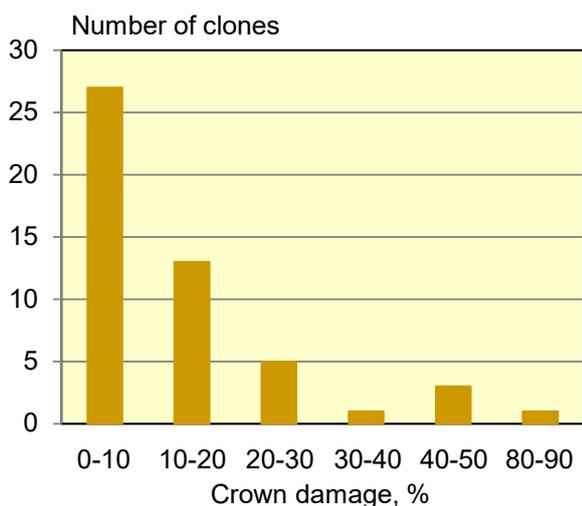
Figure 17. The number of clones in six classes of damage in an Austrian seed orchard (after Kirisits and Freinshlag 2012).

In an Austrian seed orchard with 51 clones the crown damage was studied at age 16-17 years (Kirisits and Freinschlag, 2012.) The number of grafts per clone was 4, a low number. Damage in each third of the crowns was determined and a mean value calculated for each graft. Leaf-fall was also registered. Fig. 17 shows the number of clones in the various classes of percentage damage. More than half (27) of the clones were in the class with 0–10% damage while an extremely sensitive clone had more than 80% with crown damage. Crown damage was not correlated with leaf-fall, perhaps because the mean damage level was low, 13.2%. Genetic parameters were not estimated, but the big difference in mortality within a population agrees with

other studies.

In two Lithuanian studies of ash with serious dieback (Pliura et al., 2015), the progenies were studied from 10 of the trees. Each population was represented by five offspring from individual trees. At 4 years old, a third of the material was exposed to an artificial freeze test at -5°C on 16 May and another to a drought test in the greenhouse at 25–35°C for two weeks, starting 20 June. The remaining third of the population served as control. Several characters relevant to ash dieback were involved in the investigation. Here we limit the discussion to the absolutely most important character: survival. This varied between 20% and 100% in the control material, which had the lowest mean survival, 66.9% (Fig. 18). The freeze-tested material had the highest survival, 77.0%. The differences among the various treatments were not, however, statistically significant. Fig. 18 shows also that the ranking among the progenies differs according to treatment. This is confirmed by the statistical analysis, which shows that the interaction between progeny and treatment was significant. This interaction was so strong that the difference between progenies was not significant. The limited number of trees per progeny and treatment can have made it difficult to demonstrate significant differences. In contrast, there were significant differences between progenies in the control material, with very high narrow-sense heritability, 0.69. This implies that selection for survival under normal conditions without drought stress or frost treatment can be very
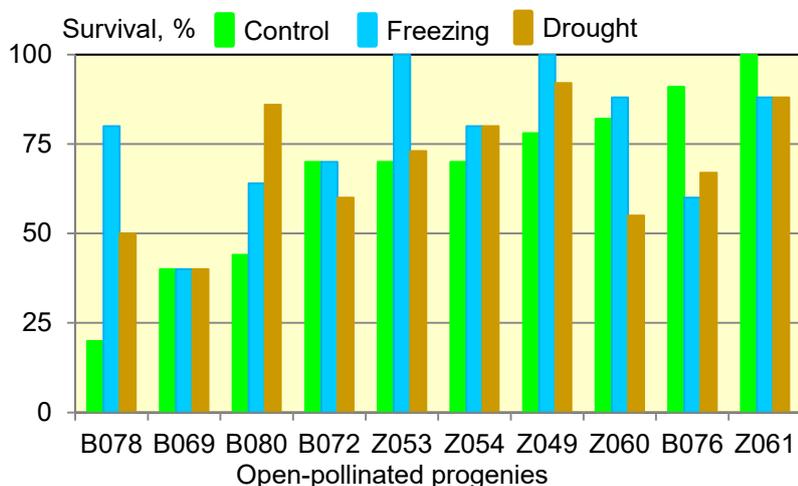

Figure 18. Percent survival in 10 open-pollinated ash families from two Lithuanian populations, Birzal and Zeimelis. One third were exposed to frost treatment (-5°C) in May. Another third were drought-stressed for two weeks starting 20 June; no watering in the greenhouse where the temperature varied from 25 to 35°C. Survival was registered 3 September (after Pliura et al., 2015).

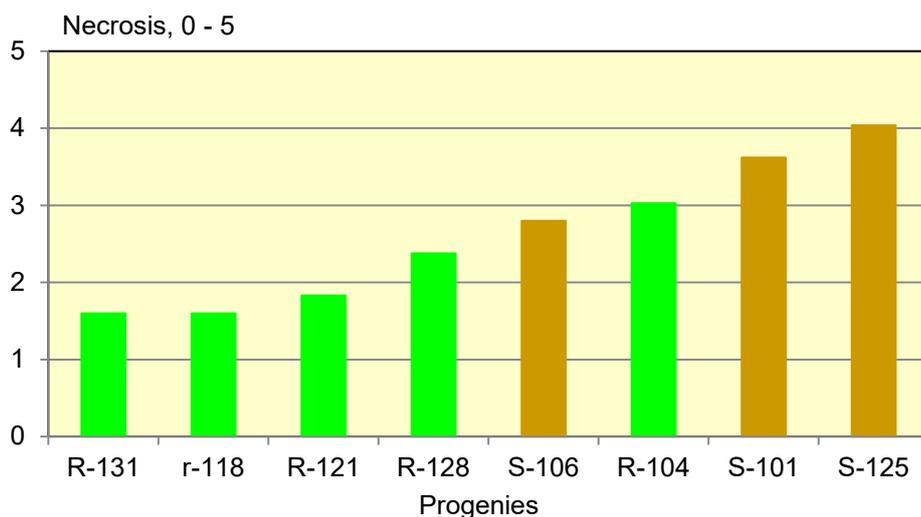successful. This investigation shows further that survival is strongly affected by the environment to which the young ash trees are exposed.

Viridiol is a phytotoxic substance that fungi use to attack a host plant. The reaction of the ash clones with varying sensitivity to *H. fraxineus* after treatment with this substance was studied in the progenies from five resistant and three susceptible clones (Cleary et al., 2014). They were treated with three concentrations of viridiol, 14.7 μmol, 147 μmol and 1.47 mmol. The most comprehensive damages were those after treatment with 147 μmol, but the differences compared with the highest dose were not great. Fig. 19 shows that one of the progenies from a resistant clone had necroses of the same size as the susceptible clones, while the other resistant clones had milder damages with a mean value of about 0.06. If viridiol plays a roll in spontaneous infection with *H. fraxineus* the results suggest that there are different mechanisms behind the resistance of the host plants.

3.   Genetic variation among populations

In Lithuania three provenance trials with 10 Lithuanian populations and 11 European populations were established (Pliura et al., 2011). Each population consisted of progenies from individual trees after open pollination. In one of the trials the survival was only 2%, which did

not allow any meaningful genetic evaluation. The other two trials enabled estimations of differences between and within populations.

The results as regards survival in this study are shown in Fig. 20. The native Lithuanian populations had a higher mean survival (45%) than that of the other populations (26%). For tree height at 8 years old there were also big differences between populations. It is worth noting that 768 of the 27,000 trees that were planted did not show signs of dieback—just short of 3%.

Even if the ranking of populations was not exactly the same in the two trials, the differences were not dramatic. The results from the one trial mirrored those in the other trial quite well.
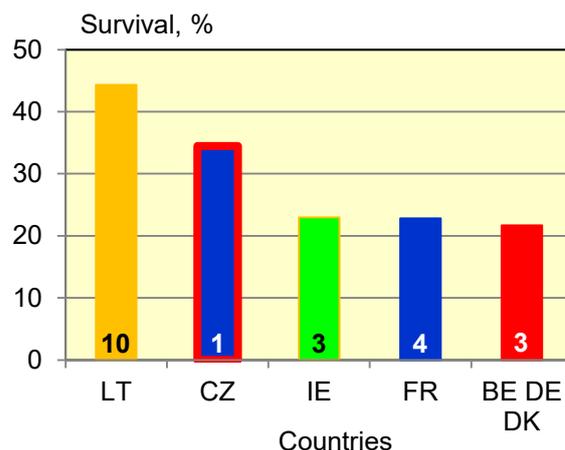

Figure 20. Survival of 21 ash populations at age 8 years trial in Lithuania. Abbreviations for the lands of origin as standard within the EU. The numbers indicate how many populations are involved in each column (after Pliura et al., 2011).
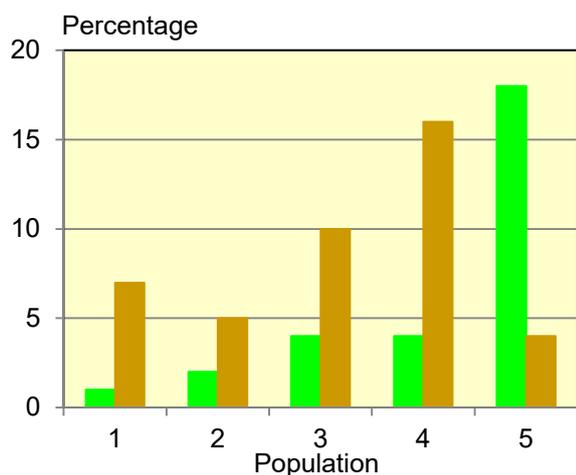
Figure 21. Percent undamaged trees (green) and dead trees (brown) in 5 German ash populations studied in 4 German field trials) after Enderle et al., 2013).
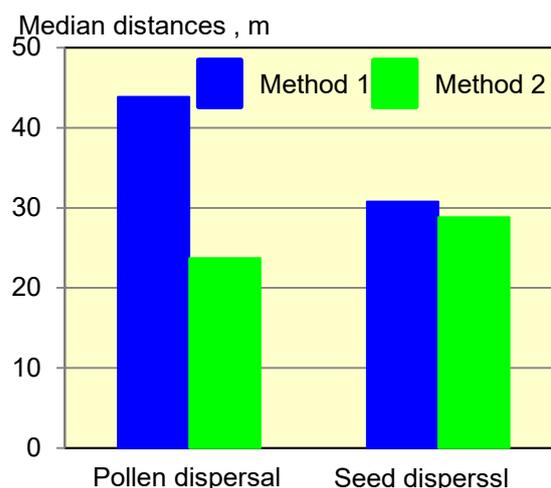

Figure 22. Median distances of pollen and field dispersal in a study of a German population. The estimates were based on 13 microsatellite loci. Two different calculatioon methods were used. (after Semizer-Cuming et al., 2017).

In Germany four provenance trials with 8 German ash populations were set up in 2005 (Enderle et al., 2013.) Since three of the populations were part of only one of the trials, the results of 5 of the 8 populations are discussed here. In 2007 when the trees were 5 years old the frequency of ash dieback was 13%; the frequency of diseased trees rose gradually to 94% in 2012. The development in Germany is in accord with what happened in Lithuania. The authors considered it likely that the spread of ash dieback had not reached its peak in 2012. Fig. 21 shows that population 5 contained a much higher percent undamaged trees and lowest mortality.

The significance of the fragmentation of ash that occurred in Europe for the regeneration of ash is very limited. A study has aimed at clarifying the crossing pattern in a German population in a stand of about 2 hectares (Semizer-Cuming et al., 2017). In the study, 13 microsatellites were used to characterize the genotypes of the trees in the stand, in the progenies of the trees in the stand, and in a planted avenue within 500–2,000 meters from the stand.
Seed and pollen dispersal within the stand were calculated by two different methods. The methods gave different results for pollen dispersal: from 0 to 939 meters with the first method and

from 2 to 340 meters with the second. Pollinations within the stand up to 100 meters were dominating. Differences were small as regards seed dispersal (Fig. 22), where the dominance of short seed dispersal was even more pronounced. All the seed was spread within 100 meters according to the first method of calculation. Inbreeding within the stand was very limited, with an inbreeding coefficient of 0.006 while for the progenies it was 0.021, which implies that a number of inbred plants had been outcompeted during the development of the stand. Pollination from the tree avenue was limited to a few percent. A stand for gene conservation should be at a distance of some hundred meters in order as far as possible to avoid contamination from planted ash and other stands.

4.   Species differences

Species that have been exposed to *H. fraxineus* for many generations in their homelands in East Asia have certainly inherited a resistance to ash dieback. Although there does not seem to have been a statistically adequate test of resistance in other ash species, some observations have been published. The investigation covering most species was carried out in the arboretum in Hörsholm in Denmark, where there is a great press
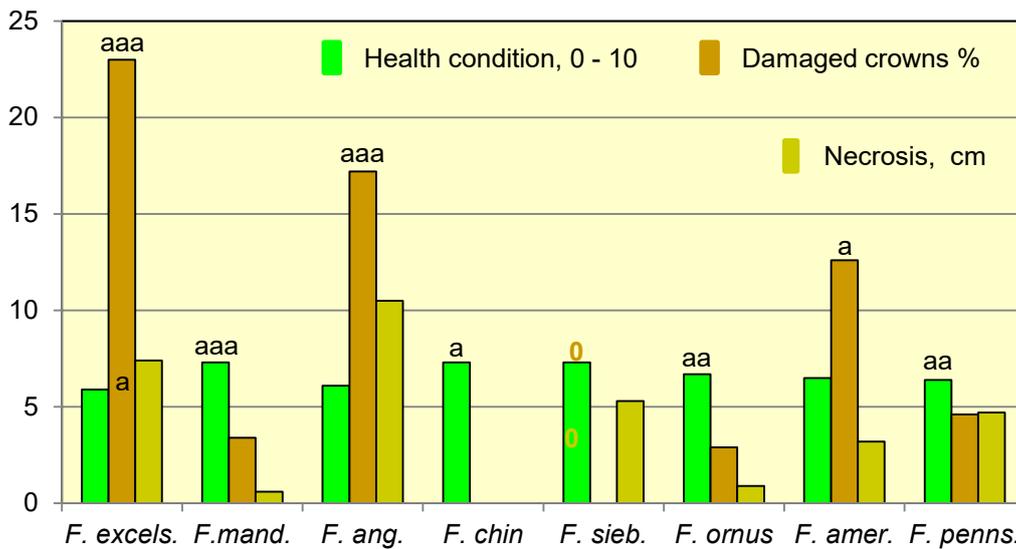
Figure 23. Health condition (0= dead, 10 = symptom-free), mean percent damage to crowns, length of necrosis in cm after inoculation with H. Fraxineus *and formation of apothecia (0 = no apothecia, a = 1–3 apothecia, aa = 4–10 apothecia, aaa = more than apothecia) in various* Fraxinus *species in a Danish arboretum in Hörsholm.* excels. = excelsior, mand. = mandchurica, ang. = angustifolia, chin. = chinensis, sieb. = sieboldianum, amer. = americana, penn. = pennsylvanica *(after Nielsen et al., 2017).*

from *H. fraxineus*. The Danish trials (Nielsen et al., 2017) indicate differences in resistance among species. In the arboretum the general state of health of the trees was registered as well as the percentage of each tree crown that was attacked by ash dieback. In addition, leaves and stems were inoculated with the pathogenic fungus; and the occurrence of apothecia after exposure to ascospores was tested for.

Fig. 23 is a summary of results regarding the general health of the trees, percentage damage in the crowns and the necrosis length after inoculation with *H. fraxineus*. There were significant differences between species for all the characters. The figure shows that the general health did not vary much among the species, but there were big differences as regards the percent damage, with the greatest percentages for *F. excelsior* and *F. angustifolia*. The East Asian species *F. chinensis* and *F. sieboldiana* were symptom-free. Twelve of the 13 trees of *F. mandchurica* were symptom-free while the thirteenth tree had mild damages from ash dieback. The American species *F. americana* and *F. pennsylvanica* lay in between. The European *F. ornus* was relatively resistant to attacks from *H. fraxineus*;

only one of the seven trees examined of this species was crown-damaged, and the damage was mild; fewer than 10% of *F. ornus* showed even limited necrosis, like *F. mandchurica* and *F. chinensis*. Apothecia were present to a greater extent in *F. excelsior, F. angustifolia* and *F. mandchurica* but were completely absent in F. sieboldiana (Fig. 23). Despite the great extent of apothecia in *F. mandchurica*, it appears that this species defends itself actively against further attacks by *H. fraxineus*. In the natural range of *F. mandchurica, H. fraxineus* is present without causing marked damage on this ash species. The positive results for *F. mandchurica* are of greatest interest for species crossing because the species belongs to the same subgenus of *Fraxinus*, so one expects that it will be easier to obtain hybrids between our ash and Manchurian ash than with *F. chinensis* or *F. ornus*, which belong to another subgenus of *Fraxinus*.

As regards our ash we know that hybridization with the closely related narrow-leaved ash, *F. angustifolia,* occurs spontaneously when they are growing nearby. There does not seem to be more information on crosses between our ash and other resistant species. As seen from Fig.
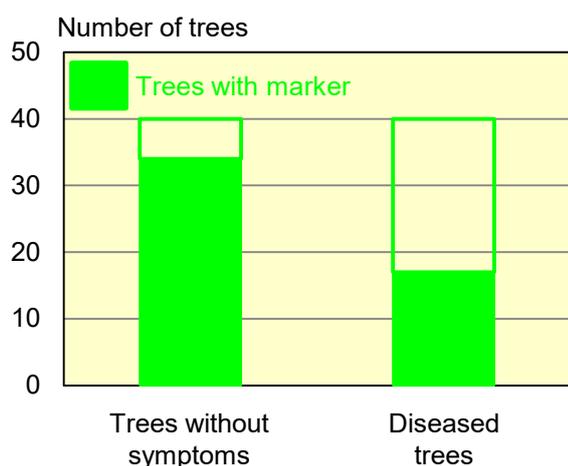
Figure 24. Number of trees with a resistance marker for symptom-free trees and diseased trees (after Menkis et al., 2019).

23, crosses with *F. angustifolia* are not to be considered if the aim is to enhance the resistance of *F. excelsior* to ash dieback.

5.   Markers

The dream of a forest tree breeder is easy identification of plants and trees that are resistant to diseases and pathogens. Several studies have aimed at identifying at gene level what leads to resistance to ash dieback. Some progress has been made and a research group at SLU has recently presented promising results (Menkis et al., 2019). For a molecular genetic study the group chose ashes with fewer than 10% damages from various localities on Gotland. These were selected 2013–2014 at sites where ash dieback was widespread. The trees were inspected again in 2017 and 2019. Samples for the molecular genetic analysis were taken from 50 symptom-free trees and 50 trees with serious symptoms of ash dieback. Based on 40 symptom-free and 40 strongly infected trees, the occurrence of a resistance marker could be securely analyzed. The results are shown in Fig. 24; considerably more of the symptom-free ashes than of the diseased trees carry the resistance marker. Since 17 of the diseased trees also carry the marker, carrying the marker is no guarantee for resistance to ash dieback. The hope is that one can identify more resistance markers so that one can finally obtain a combination of markers that clearly in-

dicate resistance to ash dieback.

If several favourable marker DNA sequences are identified in different trees and subsequently combined into one genotype, there can be problems with non-allelic interaction; a DNA sequence that is slightly positive in one genetic background, can be neutral or slightly negative in another genetic background. Furthermore, the trees intended for planting must consist of many genotypes to avoid problems arising from genetic uniformity. Traditional breeding programs are designed to minimize problems from non-allelic interaction and provide genetic variation.

Earlier a Danish/English study (Harper et al., 2016) identified three markers associated with a degree of resistance for ash dieback. One of the markers emerged also in *F. americana, F. mandchurica* and *F. ornus*, which showed resistance to ash dieback. There is therefore hope of finding a battery of markers that indicate resistance to ash dieback even if the resistance is regulated by a large number of genes.

A Swedish research group has inoculated material within a seed orchard and two-year-old plants from a commercial nursery (Sahraei et al., 2020). Two resistant and two susceptible clones in the seed orchard were inoculated with plugs of agar containing *H. fraxineus*. Tests were made 10 months after inoculation. Nursery plants were inoculated with plugs containing *H. fraxineus* in direct contact with the cambium. Damage in the nursery plants was registered on four occasions; 7–42 days after inoculation. On each occasion tests of the phloem tissue were taken to investigate gene activity in the tissue and in particular to establish which genes were expressed in the various tissues.
The length of the necrotic tissues after inoculation was 7 and 8.5 cm respectively in the two resistant clones, and considerably longer in the two susceptible clones, 32 and 70 cm respectively.
In Fig. 25 the results are summarized as regards genes with different expression in resistant and susceptible clones, some in healthy, some in necrotic tissue. As shown in the figure, a large
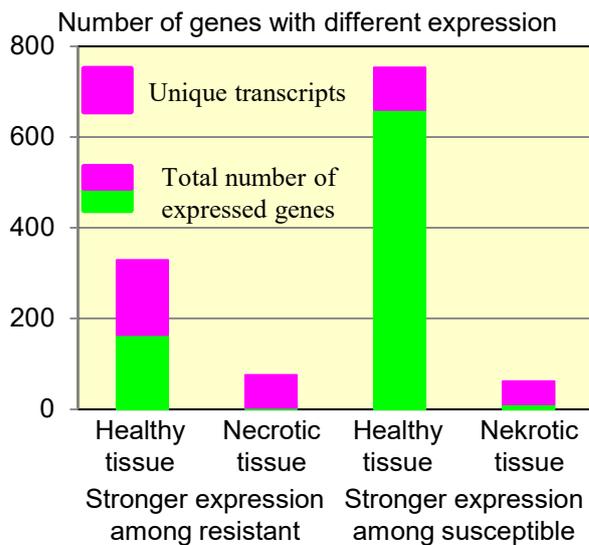
Figure 25. Number of unique transcripts and total numbers of expressed genes in resistant and susceptible ash clones after inoculation with H. fraxineus *(after Sahraei et al., 2020).*

number of genes are expressed differently in healthy and resistant clones. The differences between the two types of clones are, as seen from the figure, less obvious in necrotic tissue. In the experiment with nursery plants 204 genes were discovered with different expression in healthy from diseased tissue; 149 of these genes were induced while 55 were under-expressed. Comparison with the experiment with clones showed that 47 genes had similar expression as clones and as nursery plants.

In an investigation aimed at finding molecular markers for resistance to ash dieback, 111 phenotypically symptom-free trees and 215 susceptible trees were chosen (Chaudhary et al., 2020). Statistically significant correlations with toleration of ash dieback could be demonstrated but the explanatory powers were in all cases under 6%, which ruled out the use of these markers in breeding; but the results have increased our understanding of the building up of resistance to ash dieback.

A successful experiment by an international research group to find markers with the help of Fourier-transformed spectroscopy (Villari et al., 2018) involved bark from 76 symptom-free genotypes with known resistance to ash dieback. Resistant and susceptible genotypes could be discriminated with spectroscopy over the wavelength regions 748–798 cm^{-1} and 879–947 cm^{-1}, with the strongest discrimination at wavelength 895 cm^{-1}. This shows that a phenolic is involved in discrimination. According to the study, this phenolic is an excellent marker to distinguish resistant and susceptible ashes.

In another study of 1,250 trees (Stocks et al., 2019), 3,149 single nucleotide polymorphisms (SNP) were related to resistance to ash dieback. About 200 of them could be correlated with resistance to ash dieback. About 100 of them could be correlated with varying degrees of damage caused by *H. fraxineus*. Such a tool proved to provide a good marker for an important level of resistance to ash dieback, while another marker signified less resistance for the disease. A third was a marker for great susceptibility for ash dieback.

One of the conclusions from this study was that resistance is regulated by genes at many loci. This agrees with the expectations given in the introduction about the regulation of resistance in trees with a long generation time.

6. Genetic variation in *Hymenoscyphus fraxineus*

Parasitic fungi can also vary. A Danish group (Kosawang et al., 2020) investigated 19 strains of *H. fraxineus* and three full-sibling families of ash. The stems were inoculated with infected wooden plugs and the development of necroses measured on different occasions up to 250 days after inoculation. The stems' heredity were studied and there were found to be 19 genetically unique strains. Led by these results, 8 strains were chosen for a study of their growth on two different culture media.

As seen from Fig. 26, there was no necrosis from one of the strains while the most aggressive strain had a mean growth of 39.5 cm over the 250 days of the trial. The difference among the strains was statistically highly significant. Similarly the differences in growth among the 8 strains that were followed on culture media were highly significant. There was, however, no correlation between the results from the two types of study. Culture on artificial media appears not to be a suitable means for testing the pathogenicity on growing trees.

The great variation in aggressivity of different strains is a factor that complicates the practical breeding of ash. It is not enough to find resistant individuals to a strain in a very large tree population; such an individual has to be resistant to several other strains of the parasitic fungus.

The genome of *H. fraxineus* contains genes coding for two enzymes that break down pectins, which can explain why this fungus, unlike the closely related *H. albidus*, is pathogenic.

7. Breeding

The seed orchards established in various countries before the arrival of ash dieback aimed at improvement of ash wood quality and yield. These orchards are components of simple breeding activities. However, any long-term breeding plans are not known to us. After the arrival of
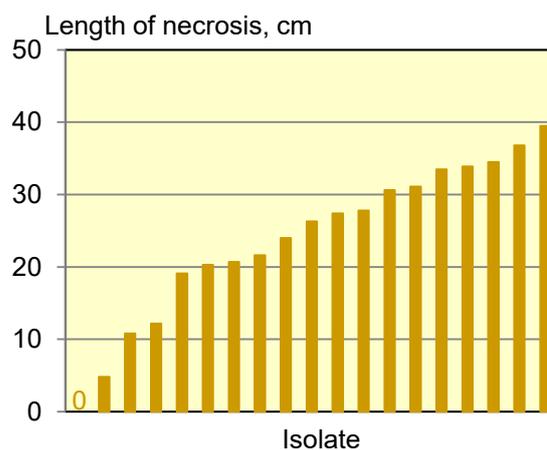

Figure 26. Length of necrosis in three full-sib families of ash after inoculation with 19 isolates of H. fraxineus*. Evaluations were made 250 days after inoculation in the stems of young ashes (after Kosawang et al., 2020).*

ash dieback, preparations for breeding are underway. A large number of symptom-free ashes have been selected in stands with comprehensive damages, and grafts have been taken from hundreds of trees. The aim is to produce grafts to use in crossings, which it is hoped will result in resistant progeny.

Flowering in future ash seed orchards is important for breeding for resistance. A study of the correlation between ash dieback and flowering in a Danish seed orchard on northern Zealland with 39 clones has been presented (Semizer-Cuming et al. 2017). The seed production of the clones was estimated on a 10-degree scale where 0 = no seed and 9 = rich production. The proportion of female flowers was estimated on an analogous 10-degreee scale. Clones that had 0–20% female flowers were classed as male clones (15 clones) while female clones had more than 80% female flowers (16 clones). The remaining clones were classed as hermaphrodites and were not included in studies of the correlation between flowering and ash dieback. Nine microsatellites were used to determine the paternity of the progenies. The percentage of trees with damaged crowns was related to the abundance of seed and to the fathers in the progeny.
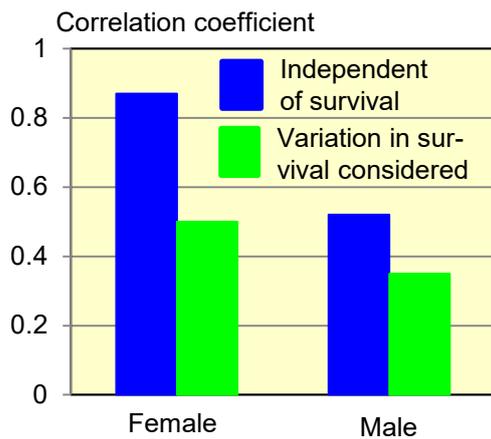
Figure 27. The correlation between female or male mating success and crown dieback, either without considering impact of variation on number of living grafts of individual clones (blue columns) or considering survival (green columns) (after Semizer-Cuming et al., 2019).

Separate correlations were calculated regarding female flower production with the total number of seeds and with the mean seed production from living grafts. The total seed production differs from the seed production per living graft if the number of living grafts varies among the different clones. Corresponding calculations were made for correlations for the male side. As Fig. 27 shows, the correlations are stronger on the female than on the male side. All the correlations were significant. An explanation can be that trees that are exposed to great stress invest more in the production of seed than of pollen. Differences in the correlations between total seed production and seed production per living graft depend on great differences in the number of surviving grafts among the various clones. A similar explanation applies to successful fatherhood. The significance of the correlations on the male side depends on one clone with exceptionally successful fertilization. No fewer than 46 of the 92 established fatherhoods were due to this clone.

8.   Genetic conservation

It is widely accepted that species conservation is of the utmost importance, as ecologists have long urged. One million species are believed to be at risk of extinction (see Hultberg et al., 2020). Species extinction is hardly a new phenomenon, as 99% of species (five billion) became extinct before the arrival of mankind (e.g., Kunin and Gaston, 1996, Jablonski 2004). Currently there is a new threat of mass extinction like that of the Pleistocene ice-age. It is not really possible to save all the threatened species. What is perhaps feasible, though costly, is to conserve keystone species; species that have great significance for the survival of other species in the ecosystem (e.g., Eriksson et al., 1993; Varela and Eriksson, 1995). Ash is a keystone species (Hultberg et al., 2020; Fig. 28). Charismatic species – species that have a special appeal to, or value for, humans – are sometimes given priority in genetic conservation (e.g., Ducarme et al., 2012). Ash is reasonably regarded as a charismatic species, even if it is rarely planted in gardens; the autumn colours of ash tree leaves are particularly attractive (cover picture). An excellent example of an uncharismatic species is *Hymenoscyphus fraxineus*, the fungus that causes ash dieback.

Ash dieback is not only a threat to the survival of ash but to species that depend on ash for their existence. For Sweden an analysis was presented (Hultberg et al., 2020) of how many species are involved and to which category they belong. Species that are totally dependent on ash for their existence are coined associated species. They run the greatest risks of extinction. Fig. 28 shows that most of these species are invertebrates or lichens. It is arguably important that the conservation

Risk for extinction, number of species

High

Moderate

Mosses Fungi Everte- Lichens
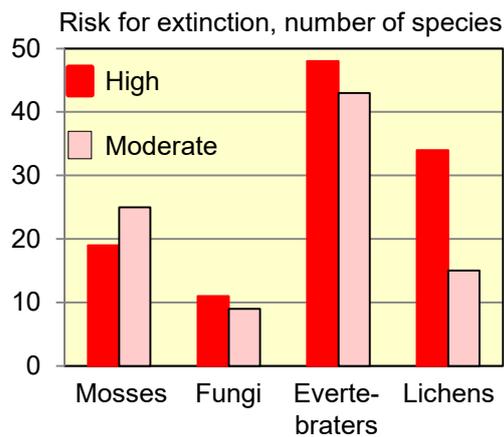   braters

Figure 28. Number of species with high risk of extinction and of moderate risk of extinction owing to ash dieback. Those with high risk of extinction are strongly associated with, but not totally dependent on, ash trees (after Hultberg et al., 2020).

of ash is organized so that the survival of these so-called associated species is guaranteed by the methods that are used.

As a keystone species that is threatened with extinction, ash should be given priority in genetic conservation.

Besides the technical guidelines for conservation of ash published by EUFOR-GEN (Pliura and Heuertz 2003) we are not aware of any other report on genetic conservation of ash. In the EUFORGEN report the so-called multiple population breeding system, MPBS, consisting of 20–30 subpopulations, was suggested for genetic conservation of ash. For a detailed description of MPBS see Eriksson et al. (1993). The subpopulations should cover the environmental conditions in the distribution area of ash in Europe. As a complement to the *in situ* MPBS a network of *ex situ* progeny plantations was suggested. It should be remembered that the EUFORGEN report was written before the large-scale death of ash had occurred. Today, 2022, it is unlikely to implement the MPBS of *in situ* conservation suggested since there are few if any stands of ash

remaining in most ecozones in Europe. For each ecogeographic zone we suggest that grafts or cuttings from resistant trees are planted together in clonal archives that will serve as seed sources for new plantations of ash. Such archives should be located such that contamination from natural ash is avoided. The management practice of ash stands imposed by the Danish Nature Agency, in which unhealthy trees are logged and healthy trees are maintained (see McKinney et al., 2014) should be followed. This strategy is supported by the finding that there is a positive correlation between ash dieback resistance and seed production (Semizer-Cuming et al., 2019), which means that resistance will be improved in each generation.

Varela and Eriksson (1995) suggested an extension of some of the MPBS subpopulations to take care of the genetic conservation of associated species. This approach can obviously not be applied for species dependent on ash for their survival. Even if all associated species will not be conserved with the suggested genetic conservation plantations we are convinced that a majority of them will be conserved by locating these plantations in different ecozones.

What is likely to happen if no conservation efforts are taken to save our ash? Numbers of ash trees will decline sharply, with danger of extinction; but they might slowly recover, with natural selection, and conscious or unconscious artificial selection, favoring the 1–3% fully resistant and a larger number with partial resistance or tolerance. However, active conservation efforts will be a surer way for genetic conservation of ash.

Perhaps ecologists will accept the replacement of diseased ash with resistant ash in natural forests and lightly managed woodland, with the help of volunteer labor. Also, we can expect regional authorities to plant resistant ash trees in town av-

enues, as an addition to Norway maple, silver birch, limes and cherry trees, just as they did before the arrival of ash dieback. The numbers required would be small by the standards of commercial forestry, and it is currently unclear who will pay for a tree breeding program.

9. Summary

The results from several studies show that the naturally occurring resistance to ash dieback is low, 1–5%. Fortunately the studies with sexually derived progenies demonstrated high heritabilities. This indicates good conditions for successful breeding for resistance to ash dieback. Further support for this conclusion come from the high estimates of clonal repeatabilities from clonal studies.

Different populations show widely varying responses to ash dieback. The limited results published to date do not suggest that any population is satisfyingly resistant. A Lithuanian study of 21 populations showed that the majority of exotic populations were greatly more damaged than the ten native populations. It appears that the import of populations is not the way to obtain resistance to ash dieback.

Crosses with trees of resistant ash species can be a way forward in ash breeding. Knowledge of different ash species' resistance to ash dieback is limited. Species that have been exposed to *Hymenoscyphus fraxineus* for a long time in their native environments have acquired resistance to ash dieback. Crosses between our ash and the east Asiatic *F. mandchurica* should be of the greatest interest.

As expected, the results indicate that many genes, mostly of small effect, regulate resistance to ash dieback. Markers for resistance have, however, been identified, which can indicate a fast track to selection of resistant trees.

A complicating factor for resistance breeding is the large number of strains of *Hymenoscyphus fraxineus*.

A large number of symptom-free ashes have been selected in stands with comprehensive ash dieback. Breeding for resistance can get off to a flying start as soon as resources for breeding are secured. How best to exploit the resistant ashes from a breeding program also needs attention.

Acknowledgments

References

Chaudhary R, Rönneburg T, Åslund MS, Lundén K, Durling MB, Ihrmark K, Menkis A, Stener L-G, Elfstrand M, Cleary M, Stenlid J (2020) Marker-trait associations for tolerance to ash dieback in common ash (*Fraxinus excelsior* L.) Forests 11, 1083; doi: 10.3390/f11101083

Cleary MR, Anderson PF, Broberg A, Elfstrand M, Daniel G, Stenlid J (2014) Genotypes of *Fraxinus excelsior* with different susceptibility to the ash dieback pathogen *Hymenoscyphus pseudoalbidus* and their response to the phytotoxin viridiol – A metabolomic and microscopic study. Phytochemistry 102, 115-125. http://dx.doi.org/10.1016/j.phytochem.2014.03.005

Ducarme F, Luque GM, Courchamp F (2012) What are "charismatic species" for conservation biologists? https://www.researchgate.net/publication/302596828

Enderle R, Peters F,Nakou A, Metzler B (2013) Temporal development of ash dieback symptoms and spatial distribution of collar rots in a provenance trial of *Fraxineus excelsior*. Eur J For Res 132, 865–876

Enderle R, Nakou A, Thomas K, Metzler B (2014) Susceptibility of autochthonous German *Fraxinus excelsior* clones to Hymenoscyphus pseudoalbidus is genetically determined. Ann For Sci 72, 183-193. https://doi.org/10.1007/s13595-014-0413-1

Eriksson G, Namkoong G, Roberds J (1993) Dynamic gene conservation for uncertain futures. Forest Ecology and Management 62, 15–37

Eriksson G, Ekberg I, and Clapham D. (2020) Genetics Applied to Forestry – An Introduction. 226 pp. https://www.slu.se/en/departments/plant-biology-forest-genetics/education/forest-genetics-online/

Harper AL, McKinney LV, Nielsen LR, Havlickova L, Li Y et al. (2016) Molecular markers for tolerance of European ash (*Fraxinus excelsior*) to dieback disease identified using Associative Transcriptomics. Scientific Reports 6:19335

Hebda A, Liszka A, Zlobicki P, Nawrot-Chorabik K (2021) Transformation of European ash (*Fraxinus excelsior* L.) callus as a starting point for understanding the molecular basis of ash dieback. Plants 2021, 10, 2524. https://doi.org/10.3390//plants10112524

Hultberg T, Sandström J, Felton A, Öhman K, Rönnberg J, Witzell J, Cleary M (2020) Ash dieback risks an extinction cascade. Biological Conservation 244, 108516. https://doi.org/10.1016/j.biocon.2020.108516

Jablonski, D. (2004) Extinction: past and present. Nature 427, (6975): 589.Bibcode:2004Natur.427..589J. doi:10.1038/427589a. PMID 14961099. S2CID 4412106.

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

Kjær ED, McKinney LV, Nielsen LR, Hansen LN, Hansen JK (2012) Adaptive potential of ash (*Fraxinus excelsior*) populations against the novel emerging pathogen Hymenoscyphus pseudoalbidus. Evol. Appl. 5, 219– 228.

Kjær ED, McKinney LV, Hansen LN, Olrik DC, Lobo A, Thomsen IM, Hansen, JK, Nielsen, LR (2017) Genetics of ash dieback resistance in a restoration context – experiences from Denmark. In Dieback of European Ash (*Fraxinus* spp.): Consequences and Guidelines for Sustainable Management. Eds. R. Vasaitis and R. Enderle. 106-114.

Kosawang C, McKinney LV, Nielsen LR, Kjær ED (2020) Variation in aggressiveness of *Hymenoscyphus fraxineus* genotypes amid the ash dieback epidemic. Plant Pathology https://doi.org/10.1111/ppa.13158

Kunin, W.E.; Gaston, Kevin, eds. (1996). The Biology of Rarity: Causes and consequences of rare–common differences. ISBN 978-0412633805.

Lobo A, Hansen JK, McKinney LV, Nielsen LR, Kjær ED (2014) Genetic variation in dieback resistance: growth and survival of *Fraxinus excelsior* under the influence of *Hymenoscyphus pseudoalbidus*. Scand J For Res 29, 519–526

Lobo A, McKinney LV, Hansen JK, Kjær ED, Nielsen LR (2015) Genetic variation in dieback resistance in *Fraxinus excelsior* confirmed by progeny inoculation assay. Forest Pathology 45, 379-387

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

McKinney LV, Nielsen LR, Collinge DB, Thomsen IM, Hansen JK, Kjær ED (2014) The ash dieback crisis: genetic variation in resistance can prove a long-term solution. Plant Pathol 63, 485-499

Menkis A, Bakys R, Åslund MS, Davydenko K, Elfstrand M, Stenlid J, Vasaitis R (2019) Identifying *Fraxinus excelsior* tolerant to ash dieback: Visual field monitoring versus a molecular marker. Forest Pathology 50(5). DOI 10.1111/efp.12572

Nielsen LR, McKinney LV, Hietala AM, Kjaer ED (2017) The susceptibility of Asian, European, and North American Fraxinus species to the ash dieback pathogen *Hymenoscyphus fraxineus* reflects their phylogenetic history. Eur J For Res 136, 59–73. DOI 10.1007/s10342-016-1009-0

Nilsson P, Roberge C, Fridman J, Wulff S (2019) Forest Statistics 2019. Official Statistics of Sweden. 2019. Swedish University of Agricultural Sciences, Umeå (ISSN 02800543, 138 pp).

Pliura A, Heuertz M (2003). EUFORGEN Technical Guidelines for genetic conservation and use for common ash (*Fraxinus excelsior*). International Plant Genetic Resources Institute

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

Pliura A, Lygis V, Marciulymiene D, Suchockas V, Bakys R (2015) Genetic variation of *Fraxinus excelsior* half-sib families in response to ash dieback disease following simulated spring frost and summer drought treatments. iForest - Biogeosciences and Forestry 9,12-22. doi: https://doi.org/10.3832/ifor1514-008

Pliura A, Bakys R, Suchockas V, Marciulyniene D, Gustiene V, Verbyla V, Lygis V (2017) Ash dieback in Lithuania: Disease history, research on impact and genetic variation in disease resistance, tree breeding and options for genetic management. Consequences and Guidelines for Sustainable Management. Uppsala, Sweden: Swedish University of Agricultural Sciences p150–165

Przybyl K (2002) Fungi associated with necrotic apical parts of *Fraxinus excelsior* shoots. Forest Pathol 32, 387–394

Sahraei SE, Cleary M, Stenlid J, Durling MB, Elfstrand M (2020) Transcriptional responses in developing lesions of European common ash (*Fraxinus excelsior*) reveal genes responding to infection by *Hymenoscyphus fraxineus*. BMC Plant Biology 20, 455. https://doi.org/10.1186/s12870-020-02656-1

Santos C, Duarte S, Tedesco S, Fevereiro P, Costa RL (2017) Expression profiling of *Castanea* genes during resistant and susceptible interactions with the oomycete pathogen of *Phytophthora cinnamomi* reveal possible mechanisms of immunity. Front Plant Sci 8:515. Doi:10.3389/fpla.2017.00515. 12pp

Semizer-Cuming D, Kjær ED, Finkeldey R. (2017) Gene flow of common ash (*Fraxinus excelsior* L.) in a fragmented landscape. PLoS 12 ONE 12(10): e0186757. https://doi.org/10.1371/journal.pone.0186757

Semizer-Cuming D, FinkeldeyR, Nuelsen LR, Kjær ED (2019) Negative correlation between ash dieback susceptibility and reproductive success: good news for European ash forests. Annals of Forest Science 76, 16. https://doi.org/10.1007/s13595-019-0799-x.

Sollars ESA, Harper AL, Kelly LJ, Sambles CM et al (2017) Genome sequence and genetic diversity of European ash trees. Nature 541, 212-215

Stener L-G (2013). Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* in southern Sweden. Scand J For Res 28, 205-216. DOI: 10.1080/02827581.2012.735699.

Stener, L-G. 2018. Genetic evaluation of damage caused by ash dieback with emphasis on selection stability over time For. Ecol. Manag. 409:584-592. doi.org/10.1016/j.foreco.2017.11.049 .s

Stocks JJ, Metheringham CL , Plumb WJ, Lee SJ, Kelly LJ, Nichols RA, Buggs RJA. 2019. Genomic basis of European ash tree resistance to ash dieback fungus. Nature Ecology and Evolution 3, 1686-1696. DOI: 10.1038/s41559-019-1036-6

Timmerman V, Børja I, Hietala AM, Kirisits T, Solheim H (2011) Ash dieback: pathogen spread and diurnal patterns of ascospores dispersal, with special emphasis on Norway. EPPO Bulletin 41, 14–20

Varela MC, Eriksson G (1995) Multiple gene conservation. Silvae Genetica 44, 28–37

Vedel H, Lange, J (1965) Trees and Bushes in Wood and Hedgerow. Methuen: London, 224 pp.

Villari C, Dowkiw A, Enderle R, Ghasemkhani M et al. (2018) Advanced spectroscopy-based phenotyping offers a potential solution to the ash dieback epidemic. Nature Scientific Reports 8, 17448 DOI:10.1038/s41598-018-35770-0

Woodcock P, Marzano M, Quine CP (2019) Key lessons from resistant tree breeding programmes in the Northern Hemisphere. Ann For Sci 76:51. Doi.org/10.1007/s13595-019-0826-y. 16pp

Some of the 10% ash dieback resistant *Fraxinus excelsior* trees in a central Lithuanian progeny trial at Kedainiai, Photograph Alfas Pliura.

BioCenter
Department of Plant Biology, SLU
Box 7070, 750 07 Uppsala
Sverige