ResEsche – Research for survival of common ash in Germany

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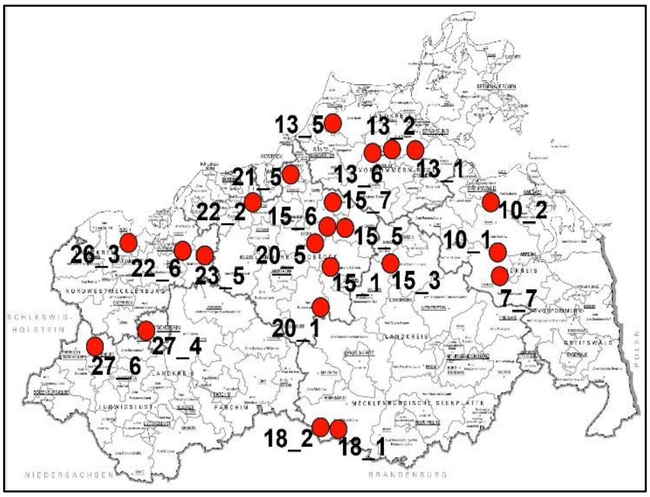
- **The survival of common ash (*Fraxinus excelsior* L.) is threatened by ash dieback caused by the fungus (*Hymenoscyphus fraxineus*) throughout Europe.**
- **Plus trees with high vitality and resistance were selected in Mecklenburg-Vorpommern.**
- **A seed orchard was established with grafts of 126 resistant plus trees. This orchard is the basis for the production of forest reproductive material with increased resistance.**

Background and aims

Ash shoot dieback caused by the fungus *Hymenoscyphus fraxineus* is progressing with undiminished aggressiveness. The associated economic and ecological damage is particularly serious in areas, where ash is often the dominating tree species on wet sites. Therefore, ash plants with a high (genetically determined) resistance to the fungus are needed in sufficiently large numbers for the reforestation of damaged forests.

A very small number of ash trees (approx. 1 to 2%) have this genetically determined and heritable increased resistance to the fungus. Based on this knowledge, this research project was launched as a joint project of the State Forestry of Mecklenburg-Vorpommern and the Johann Heinrich von Thünen Institute (Institute of Forest Genetics). The aim of the project was to establish a genetically diverse seed orchard with graftlings of such trees. This orchard will be the basis for the production of forest reproductive material with increased resistance.

Figure 1: Distribution of plus trees selected in Mecklenburg-Vorpommern



Source: Past et al. (2021)

Methodological approach

Staff of the partner State Forestry Mecklenburg-Vorpommern have selected 361 plus trees of high vitality in forest stands strongly affected by ash dieback (figure 1). These selected plus trees were characterized molecularly and phytopathologically.

For the molecular genetic detection of the fungus, the corresponding microsatellite markers were developed and applied.

Figure 2: Petioles of previous year with fruit bodies of *Hymenoscyphus fraxineus* in July 2018 (left) and leaves with necrosis in September (right)

The procedures for the vegetative propagation of ash trees were optimised. From selected plus trees, grafts were produced in sufficient numbers for the planned seed orchard. At the same time, tissue culture methods were further developed and plants were grown as rootstocks for grafting.

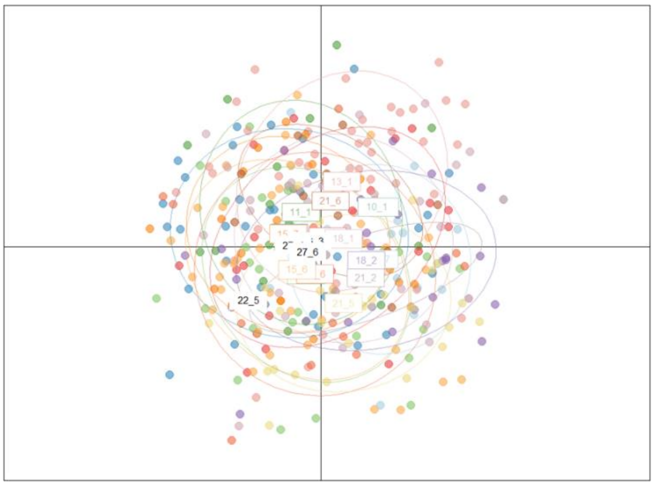
Resistance testing of the grafts was carried out in a two-stage procedure. For the ascospores test, leaf spindles (petioles) of infected ash trees were spread between the potted grafts in summer of the year of grafting (figure 2). Fruiting bodies (asci) of the fungus developed on these leaf spindles, which led to a high infestation pressure by ascospores. For the wood chip test, wood chips, which had previously been overgrown by the fungal mycelium on culture medium, were inserted into the bark of

four grafted plants per clone in late summer. These plants were overwintered in the greenhouse. The assessment of a possible fungal infestation of the shoot (dieback) was carried out in spring of the following year.
In addition, seeds were harvested from 64 of the plus trees and sown in the nursery. After two years growing in the nursery these plants should be planted into field trials for progeny testing. These trials can provide valuable insights into the inheritance of possible resistance.

Results

A protocol for molecular genetic analysis of microsatellite markers was developed and used for ash successfully. It includes three primersets with 16 pairs of primers. On the level of these markers, no clear population differences between the investigated ash stands in Mecklenburg-Vorpommern could be revealed (figure 3). Therefore, it was possible to establish one single large seed orchard with all selected and propagated genotypes.

Figure 3: Principal component analysis for 408 trees from 18 ash subpopulations gave no indication of population differences in Mecklenburg-Vorpommern (points of the same colour represent trees from the same subpopulation)



New and further cultivation of various isolates of *Hymenoscyphus fraxineus* were carried out. These cultures served as basic material for molecular genetic investigations of the fungus and for resistance testing. Detection of the pathogen in the tissue of symptomatic leaves was possible using real-time PCR.
Grafted plants were produced from 152 plus trees. The total number of grafts was 3487, with an average grafting success of 73 %. Copulation and honeysuckle grafting of scions with terminal buds in March in the greenhouse have proved successful as propagation methods. In about half of the grafts, plants from tissue culture (from resistant source trees) were used as rootstocks.

The resistance of the grafted trees (ramets) was tested by means of ascospores and wood chip tests. As a result of the resistance tests, 26 plus tree clones could not be included in the seed orchard due to increased susceptibility to ash dieback.
Within three years, 1159 grafted trees were planted on an area in Mecklenburg-Vorpommern for the seed orchard (figure 4). A total of 126 genotypes are represented in this plantation. The number of individuals is between 5 and 15 per genotype.
For the in vitro propagation of resistant clones, different culture media were tested and modified. About 2150 plants of 23 clones were transferred into soil and partly used as rootstocks for grafting. All in all, the in vitro propagation of ash proved to be difficult, especially with older donor trees.
Extensive paternity analyses were carried out on five of the 64 progenies of the plus trees. Several large full-sib families (same parent trees) were identified. These families will be used in future projects for intensive molecular genetic analysis (e.g. detection of marker genes for resistance).
Three field trials were set up with the offspring of the plus trees for progeny testing. The trails are designed in such a way that they can later be used as seedling seed orchards. Survival rate after the first summer was good with 95 and 88 % respectively.

Figure 4: The ash seed orchard Tressow in spring 2022

Further information

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Partners

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