

## Slutrapport

**Projektrubrik:** Improvement of forest reproductive material for ash: characterizing the resistance against ash dieback (Askskottsjuka)

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**Projektets löptid:** 2016-04-01 – 2018-08-31

### Summary

Over the last two decades common ash (*Fraxinus excelsior*) has been threatened by an alien invasive fungal pathogen (*Hymenoscyphus fraxineus*) introduced from Asia. The rapid spread and intensification of the disease has resulted in a serious population decline; since 2010 ash is a Red-listed species in Sweden and since 2015 its status has worsened to become 'critically endangered'. This is concerning not only for the loss of this important noble broadleaved tree species, but also for the biodiversity of species dependent on ash. Large variation in susceptibility to the pathogen has been observed within natural populations; with less than 5% of trees showing disease resistance. Studies have shown that susceptibility to damage is a heritable trait that is genetically controlled, and that considerable gain can be achieved through selection and breeding.

The aim of the work is to support the development of a more resistant ash population for planting in Sweden. To achieve this, we have initiated two projects that use both traditional selection and modern phenotyping techniques to characterize the resistance in ash.

The funded work consisted of two sub-projects: one aimed at classical selection and propagation for resistance breeding, and the other to test modern phenotyping technologies that can potentially identify resistant ash genotypes and thereby expedite the process of tree selection in practice.

In the first project, we established two additional field trials at Snogeholm using propagated selections of wild genotypes. In the second project, we used a state-of-the-art chemical fingerprinting technique known as Fourier-transform infrared (FT-IR) spectroscopy and chemometric modelling on a unique collection of material acquired from genetic field experiments across Europe, to distinguish between resistant and susceptible ash genotypes. Using a soft independent modelling of class analogy model built with infrared spectra of phloem phenolic extracts, we discovered that spectra from the mid-infrared of phloem extracts correctly predicted the tree phenotype. This model was validated across large populations of ash in Europe.

These work in the first project was a critical first step to enable targeted genotypes to be selected for further commercial propagation, breeding and possible future establishment of new seed orchards. The results from the second project suggest that modern phenotyping technologies can provide a promising approach for identifying disease resistance and can drastically advance the efficiency and timing of selecting genetically resistant ash trees, thus potentially, expediting our current selection and screening protocols for resistance breeding.

Collectively, the results of both projects will be extremely important for the restoration and sustainable management of this important noble broadleaved tree species in Swedish forests, cities and other urban and natural landscapes.

## Results

### PROJECT 1

During 2016 and 2018, three field trials were established at Snogeholm with 2-year-old ash tree clones (grafted plants propagated with scions) of healthy, vital, mature *F. excelsior* trees. The trees were previously selected based on extensive surveys conducted between the years 2013 and 2015 in forests and the natural landscape including key habitat areas for ash (nyckelbiotop) and known seed stands throughout the natural distribution range of ash in the southern half of Sweden. In those surveys, more than 500 vital ash trees were identified and marked for selection and further monitoring. A total of 65 clones were planted in 2016, 65 clones in 2017, and 42 clones in 2018. Periodic health assessments were conducted on all trials.

### PROJECT 2

Fourier Transform (FT-) IR spectroscopy shows great potential for non-invasive measurement of quality parameters that are important in disease resistance. In order to evaluate the usefulness of this technique for future use in practice, we conducted large scale testing on populations of European ash where the genetic, inheritable resistance of individuals against damage by *Hymenoscyphus fraxineus*, is known based on field evaluations conducted over several years. Consequently, our goal was to determine the feasibility and efficacy of FT-IR to phenotype European ash for resistance to ash dieback.

We collected phloem and leaf samples from *F. excelsior* trees with known susceptibility to *H. fraxineus* in six European countries: Austria, Denmark France, Germany, Lithuania, and Sweden, in collaboration with several colleagues in each of those countries. Source material originated from genetic trials established as either clonal seed orchards or for testing ash provenance or progeny.

Sample collection was performed between May and June. At each site, a minimum of three and up to eight genotypes, were selected per susceptibility class (low, intermediate and high susceptibility) based on a relative measurement of dieback intensity as determined in previous assessments. In the case of clonal trials, between two and three ramets per clone were sampled. From each individual, the current year's shoots were harvested. At the base of each leaf, sections of phloem consisting of outer bark, cortex and some cambium were dissected from the stem with a sterile razor blade, and similarly labelled and stored on dry ice in the field. All samples were then transported cold to SLU laboratory at Alnarp for further processing. In the lab, phloem and leaf tissues were finely ground in liquid nitrogen and stored at -80°C. Chemical extracts of samples were obtained by adding 700 µl of 70% acetone, 30% water to each tube. Samples representing the extreme susceptibility groupings of *F. excelsior* genotypes, resistant and susceptible, were analyzed on FT-IR spectrometer. Spectral data were displayed in terms of absorbance and viewed using Win-IR Pro Software, and then analyzed using a multivariate classification software for the selective differentiation and identification of the target classes (susceptible vs resistant). Soft independent modeling of class analogy (SIMCA), a well- developed and accepted pattern recognition method in IR spectroscopic analysis was used to identify variables important to discriminate between susceptible or resistant individuals.

We built the chemometric model using the FT-IR spectra of the twig phloem samples. We used Coomans plots and 3D class projection plots also to identify outliers, which were removed from the model. The SIMCA calibration model that best discriminated between resistant and susceptible ash trees was a 3-factor model obtained by including spectral regions from  $\sim 748$  to  $798 \text{ cm}^{-1}$  and from  $\sim 879$  to  $947 \text{ cm}^{-1}$  wavenumber. We validated our SIMCA model with the testing data set, and 100% of the ten resistant and six susceptible ash ramets randomly selected from each of the six European countries were correctly identified as belonging solely to their phenotype group. Thus, these results show that European ash possesses readily exploitable levels of resistance that can be detected using FT-IR spectroscopy. This work represents a major advancement in the application of marker-assisted technology for tree breeding and offers a novel solution in the fight against ash dieback.

## Aims

The project goals and activities were achieved according to plan within the project period.

Funding support has permitted important the start of the basis by which we will develop a more resistant population of ash for planting in Sweden. Additional genotype assessments will occur during subsequent years (with support from Skogforsk) and a final ranking of clone performance (health) will be made for further selection. A large number of deliverables related communications with stakeholders, the public and the scientific community have already been completed. Further information dissemination and extension activities centred around these established trials are planned in the coming years.

The research also proved that FT-IR spectroscopy can significantly accelerate the process of selecting resistant phenotypes and limit the need for growing out large segregating progenies as in conventional breeding programs. This technique can be applicable at a landscape level for screening large naïve populations for disease resistance, at dramatically reduced costs than traditional selection. These findings also opens the possibility for the development/refinement of advanced instrumentation for chemical metabolite profiling to be used in practice (e.g. a portable device that would allow for in situ analysis of specific plant traits in standing forest trees).

## Communication

Selected communications during the project period (more included in attached report)

### Manuscripts:

- Villari, C., Dowkiw, A., Enderle, R., Ghasemkhani, M., Kirisits, T., Kjaer, E., Marčiulyrienė, D., McKinney, L., Metzler, B., Rostgaard Nielsen, L., Pliūra, A., Stener, L-G., Suchockas, V., Rodriguez-Saona, L., Bonello, P., and Cleary, M. 2017. Advanced spectroscopy-based phenotyping offers solutions to the ash dieback epidemic. *Nature Scientific Reports* [submitted]
- Skovsgaard, J-P., Wilhelm, G-J., Thomsen, I.M., Metzler, B., Kirisits, T., Havrdová, L., Enderle, R., Dobrowolska, D., Cleary, M., Clark, J. 2017. Silvicultural strategies for *Fraxinus excelsior* in response to dieback caused by *Hymenoscyphus fraxineus*. *Forestry* 2017; 90, 455–472, doi:10.1093/forestry/cpx012
- Cleary, M., Nguyen, D., Stener, L-G., Stenlid, J., and Skovsgaard, J-P. 2017. Ash and ash dieback in Sweden: A review of disease history, current status, pathogen and host dynamics, host tolerance and management options in forests and landscapes. In: “Dieback of European Ash (*Fraxinus* spp.) -

Consequences and Guidelines for Sustainable Management. Eds: Vasaitis R, & Enderle R pp. 195-208.