

Ash dieback is one of the most dangerous epidemic diseases of *Fraxinus excelsior*. It was first observed in Poland in 1992. It has spread since then and has now been reported from more than 25 European countries. The disease is caused by the invasive, ascomycetous fungus *Hymenoscyphus fraxineus* (*Chalara fraxinea*). The pathogen was introduced into Europe from East Asia, where it occurs on *Fraxinus mandshurica* Rupr. and *F. chinensis* ssp. *rhynchophylla*, apparently without causing disease symptoms. In Europe the disease leads to the death of single trees and of whole stands, particularly stands of young trees. So far, no effective control measures have been developed for protection of ash against *H. fraxineus*. The development of successful control measures against invasive *H. fraxineus*, which is a huge threat to the survival and distribution of *F. excelsior*, is the main goal of the science. The scientific hypothesis assumes that during the pathogenic phase of *H. fraxineus* in living ash leaves (from July until leaf fall) and during the saprotrophic phase of the pathogen in the fallen leaves (from leaf fall until fructification in the following summer) some fungi may inhibit its growth and development. They may contribute to the natural restriction of disease spread.

The main aims of the project are the detection, isolation and identification of the potentially large spectrum of fungi from diseased ash leaves and the selection of fungi with potential for biological control of *H. fraxineus*. Antagonism towards *H. fraxineus* is expected to be accomplished through competing for resources and the ecological niche, inhibition direct migration of *H. fraxineus* from nervation system to shoots, production of antibiotic metabolites or parasitism.

The study will include different species of endophytes isolated from living ash leaf rachises and saprotrophs isolated from rachises of fallen leaves in the ground litter. *Hymenoscyphus fraxineus* produces apothecia with ascospores on the fallen leaves in huge numbers. In early summer ascospores begin to infect the young, developing ash leaves. Classical methods (based on morphology and sporulation in culture) and molecular methods (sequencing of ITS1/2 rDNA) will be used for identification of fungi. If necessary, sporulation will be induced by different incubation conditions in a phytotron, including application of light at different wavelengths and fluorescent light. The results of molecular analysis (fungal DNA sequences) will be deposited in GenBank. Evaluation of the trophic properties of the most common fungi will be based on tests on ash trees *in situ* (in experimental plots in the field) and *in vitro* (in tissue cultures of *F. excelsior* in a phytotron). Mycological analysis of leaves from species of ash not native to Europe (particularly *F. mandshurica* grown in an arboretum and *F. pennsylvanica* from forest or green urban areas) will be made for comparison with *F. excelsior*. Important tasks will include studies on fungal interactions. Effects of fungi on growth of *H. fraxineus* will be studied *in situ*, and in dual cultures of pathogen and test fungus *in vitro*, on malt agar. Further analysis of antagonistic interactions with *F. excelsior* will be made. The important scientific aim of the project is to define and characterize the mechanism of antibiosis (secondary metabolites). For scientifically proven cases of mycoparasitism, the *in vitro* and *in situ* experiments will be carried in order to verify these interactions.

The proposed research is expected to help provide the basis for practical biological protection of ash against *H. fraxinus*. Similar studies have been very helpful in developing the basis for protection against other broadleaved tree pathogens in Europe.