

# TreeHarm: Development of diagnostic and treatment methods to identify and protect forest trees against harmful organisms

Kiran Singewar and Matthias Fladung  
Thünen-Institut für Forstgenetik, Sieker Landstr. 2, 22927 Großhansdorf

Kontakt: Thünen-Institut für Forstgenetik, Großhansdorf  
kiran.singewar@thuenen.de, matthias.fladung@thuenen.de

## Background and aims

The federal state Schleswig-Holstein (SH) possesses the least forested area compared to the other German federal states. Besides, rising temperature causes drought stress, which may alter the seasonal synchronization between pathogens and their host plants, resulting in the organism outbreak causing huge damage to the forest trees and ecosystem.

Therefore, the project aims are to identify total fungi, bacteria, and insect community habitats in trees growing in SH using high-throughput next-generation sequencing (diagnosis). In parallel, two treatment strategies are being developed and tested for their practicability: mycorrhiza and RNA interference (RNAi) vaccination.

The diagnosis and treatment strategies will help to protect our native forest trees.

## Progress and initial results

### Sampling sites

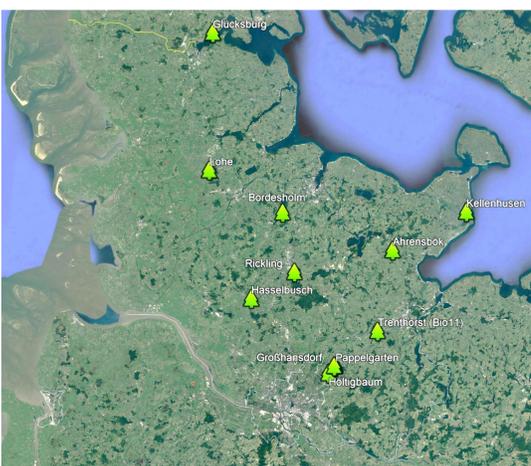


Figure 1: Different locations of sampling within the federal state Schleswig-Holstein. Leaves of a minimum of five biological replicates per tree species were collected from each site for various analyses

### Diagnosis

#### Fungal, bacterial, and insect community

Microbiome analyses are performed to evaluate the diversity of fungi and bacteria from the leaves of 150 trees sampled from different sites. The example result shows the community clustering (Fig. 2) and relative abundance of taxa (Fig. 3) in the microbiome of Norway spruce. Insect diagnosis is in progress.

## Methods

### Sampling strategies

Leaf samples of Norway spruce, Oak, and Aspen were collected from eleven different locations of Schleswig-Holstein (Fig. 1).

### Diagnosis strategies

Fungi: Internal transcribed spacer (ITS) amplicon sequencing

Bacterial: High throughput metagenome sequencing

Insect: Morphologically and barcoding of cytochrome c oxidase 1 (COX1) gene region.

### Treatment strategies

Mycorrhiza vaccination: Six mono cultures of mycorrhiza are being used to test their effect on plant development and fitness.

RNAi: Double-stranded RNA specific to vital genes is being/will be tested for RNAi-mediated vaccination to control, the Gypsy moth, Asian long-horn beetle, Poplar rust, and Ash dieback fungi.

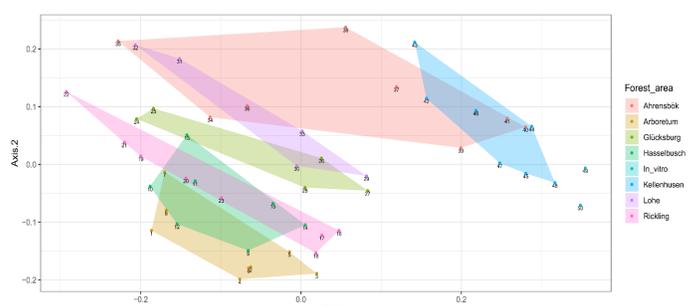


Figure 2: Community clustering of the mycobiome of Norway spruce trees collected from different locations

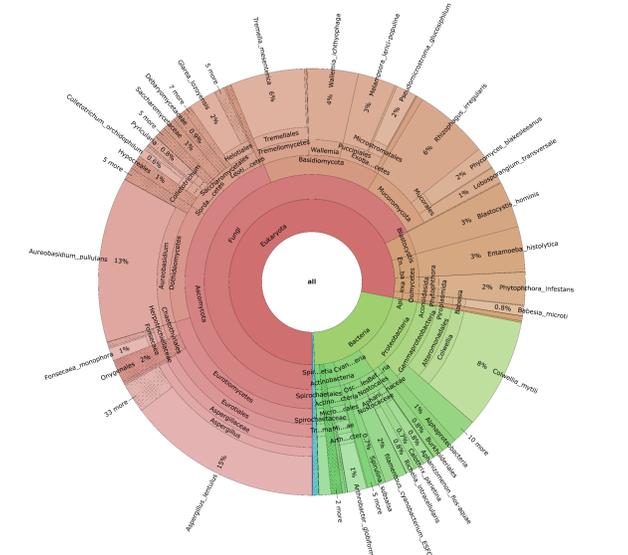


Figure 3: The relative abundance of the taxa in the microbiome of Norway spruce

## Kernaussagen des Projekts

### Diagnose- und Behandlungsstrategien können beitragen, unsere Forstbäume zu schützen

- ✓ Proben von Blättern (Eiche, Aspen) und Nadeln (Fichte) wurden von verschiedenen Wäldern aus Schleswig-Holstein geerntet.
- ✓ Pilz-, Bakterien-, und Insektengemeinschaften wurden in Mikrobiomanalysen bestimmt.
- ✓ Eine Mykorrhizaimpfung wurde mit sechs Einzelkulturen von Mykorrhiza durchgeführt, um im Feld ihre Wirkung auf Pflanzenentwicklung und Fitness zu prüfen.
- ✓ Die RNA-Interferenz- (RNAi-) Technik wird zur Bekämpfung von phytopathogenen Pilzen und Insekten getestet.

## Treatment

### RNAi-mediated and Mycorrhiza vaccine

Different dsRNA targeting vital genes of the Gypsy moth, *Lymantria dispar*, were tested on the third instar larvae. Feeding of dsRNA did not show any effects. Topical application of dsRNA Ribosomal protein S13e (Rsp15) showed a significant reduction in the larvae body weight and development followed by Chitin synthase A (CHSA), while Proteasome subunit beta 5 (PSBT5), and combinations did not show any effects (Fig. 4). Various experiments are in the progress. The mycorrhiza vaccine is being applied every six months to around 600 young trees in the field in two different experimental trials. Plant phenotyping will be performed in the next years to observe the effect of mycorrhiza vaccination on the trees.

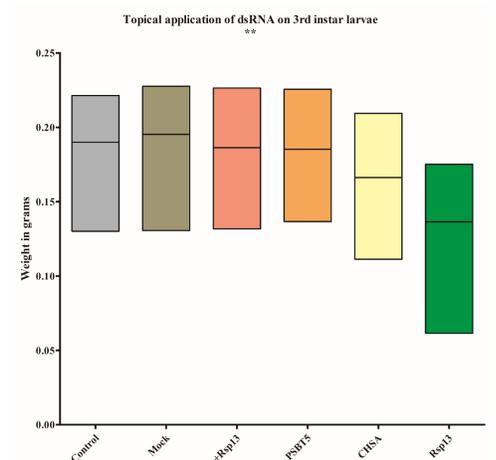


Figure 4: Delayed development in dsRNA-treated larvae compared to combo and control