Rapport of the master thesis " The reaction zone as a defense response in Norway spruce *(Picea abies)* when infected by the white-rot fungus *Heterobasidion parviporum:* differentially expressed genes and chemical changes"

Summary of the project and results

Heterobasidion parviporum (Fr.) is a pathogenic white-rot fungus that causes root and butt rot in Norway spruce (*Picea abies* (L.) Karsten), and is an extensive problem in Norway and the rest of Europe. Sapwood in 60- and 23 year old spruce trees in two separated experiments were treated with wounding or inoculated with *H. parviporum* or methyl jasmonate mimicking infection from the heartwood and inducing a reaction zone. Chemical and gene expression analysis was conducted on samples from the experimental inductions of chemical reaction zone around the inoculation and wound in the sapwood.

Secondary metabolites were identified with HPLC analysis. E-Astringin, iso-rhaphontin and one chlorogenic acid derivative decreased in concentration upon pathogen inoculation and wounding in experiment 1 and 2, respectively. Unidentified phenolic compounds (UPC) decreased upon either wounding or pathogen inoculation, or both. Gene expression was quantified using qRT PCR analysis. This vertified an induction of genes connected to the phenylpropanoid pathway. CCoAOMT1, CCoAOMT2, HCT, DAHP2, ANH2 and PAL2 were up regulated upon pathogen inoculation where inoculation depth differed in experiment 1. Here MYB8 showed differences in expression between the inoculation depths. Wounding caused an up regulation of PAL2 and CCoAOMT1 in experiment 2, while a down regulation of STS. PAL2 was up regulated upon methyl jasmonate inoculation.

The decrease of stilbene glycosides can be connected with the down regulation of STS. We hypothesize that this decrease is a degradation of the stilbenes, which in turn are converted to other compounds that may have inhibitory effects on pathogen infection. With no comparable results we are unable to conclude if the decrease of chlorogenic acid derivatives concentration are suggestive for Norway spruce when infected or

wounded. The up regulation of DAPH2 and ANH2 may be connected to the reallocation of carbon to the reaction zone, while the increased expression of PAL2 catalyzes the phenylpropanoid pathway, where HCT, CCoAOMT1 and CCoAOMT2 are involved, and indicates an increase in defense related genes upon infection or wounding in sapwood of Norway spruce, and to some degree also by inoculation of methyl jasmonate.

Evaluation of the project

The project shows the need for additional research where a higher number of ramets are used within each clone. The statistical results' weaknesses became evident during the final calculations, and an advantage would have been to combine the clones to achieve more trustworthy patterns of change in regards to gene expression as well as chemical changes. However, the project did indeed give us more information about how future projects need to be designed in order to obtain correct data.

Pictures from the fieldwork