1. Purpose of the STSM;

During the stay in the SLU from the 14th April to the 9th May 2014, in the campus of Alnarp, Sweden, I worked on two objectives of my PhD:

Objective 1: Isolation and identification of the antifungal compounds found in the extracts of the endophytes that reduce the necrosis produced by pathogens in pines.

During December of 2011, we performed several experiments that consisted of artificial inoculations of the pathogen *Gremmeniella abietina* in *Pinus halepensis* seedlings. In these plants, we also inoculate at the same time several extracts of fungal endophytes (biological antagonists). Six months later, the seedlings were revised to observe the symptoms and carried to the lab to measure the necrosis produced by the pathogen. The presence of some of the extracts in the seedlings reduced the necrosis produced by the pathogen, so we decided to analyze the compounds that were present in these extracts.

Objective 2: Identification of phenol compounds present in *Pinus halepensis* seedlings inoculated with *Gremmeniella abietina*.

In December 2012, we inoculated the pathogen *G. abietina* in seedlings of Aleppo pine from different provenances. Several months later, we evaluated the mortality of the plants and the symptoms of the disease. After that, the seedlings were cut and brought to the lab in order to measure the necrosis produced by the pathogen. Furthermore, some of the plants were freeze-dried and ground into powder in order to extract the phenol compounds. During my stay in Alnarp we measured the chemical response of the plants in terms of phenol content.

2. Description of the work carried out during the STSM;

Objective 1: Isolation and identification of the antifungal compounds found in the extracts of the endophytes that reduce the necrosis produced by pathogens in pines.

Once the fungal extracts were isolated, we put them in the RP-HPLC (Reversed Phase High-Pressure Liquid Chromatography) apparatus to separate and identify the compounds. We used a column Agilent C18 and as solvents CH_3OH and water (mobile phases). Each sample needed a 30-minute-period to complete the analysis. Since some of the samples did not show good results in the first round, we diluted those samples

in water, and after that we analyzed them again with the RP-HPLC. After that period of time, we observed the chromatogram obtained and the peaks that appeared in the chromatogram. We compared the retention time of every peak and the profile with the database of known compounds in order to identify them.

Objective 2: Identification of phenol compounds present in *Pinus halepensis* seedlings inoculated with *Gremmeniella abietina*.

The seedlings were freeze-dried, ground into powder and stored in Eppendorf tubes. After that we added 1000 µl of methanol to each sample, and put them in the shaker for 6 minutes. Later, we put the tubes in the centrifuge for another 6 minutes at maximum speed and then we removed 800 µl of the liquid of every sample and transferred it to another tube. We added another 800 µl of methanol to every sample and we repeated the process again: 6 minutes in the shaker and 6 minutes in the centrifuge. Then we put the samples in the centrifugal evaporator device (Speed Vac) for 2h to evaporate the entire methanol. After 24 hours, we added 800 µl of methanol to every sample and then we passed them trough a filter with a syringe. After that, we placed the samples in HPLC tubes, and carry them to the RP-HPLC apparatus. The conditions of the HPLC analysis were the same than the ones previously described for objective 1: we used a column Agilent C18 and as solvents CH_3OH and water (mobile phases). Once the process was completed we observed the chromatogram to find out which compounds were present in the samples.

3. Description of the main results obtained.

Objective 1: Isolation and identification of the antifungal compounds found in the extracts of the endophytes that reduce the necrosis produced by pathogens in pines.

Five samples of fungal extracts were analyzed with the HPLC. They belonged to the species: *Trichoderma*, *Aurebasidion pullulans*, *Aureobasidion* sp., Unknown deuteromycete and finally the control sample. We found some peaks at different wavelengths that mean that there were some compounds. For most of the samples we found peaks at 214nm (a wavelength which most organic compounds absorb) and at 280 nm. We are still processing and analyzing these results, but we think that some phenolic compounds were present in the samples.

Objective 2: Identification of phenol compounds present in *Pinus halepensis* seedlings inoculated with *Gremmeniella abietina*.

A total of 360 samples were analyzed with the HPLC. In most of the samples we found peaks at 214nm and 280nm wavelength. We are still analyzing these data but our preliminary results suggest that the compounds were lignins, tannins, phenolic acids/lignans and monoterpenes. We are currently determining the amount of each compound in every sample and the nature of every compound.