developed to understand its role during fungal development *on planta*. The aim of our work is to identify and to highlight the role of the genes involved in the transsulfuration pathway (cysteine ↔ homocysteine), and then for methionine synthesis from the available entire genome of *M. grisea* using genetic and biochemical tools. To this end, we create null mutants of some genes to elucidate their respective physiological role. Trophic characterizations of these mutants were developed onto minimal medium in presence of various sources of sulfur compounds. In addition, quantitative PCR analyses were performed to follow the expression of the identified sulfur genes in the three mutants by comparison to the wild type strain during fungal development and the infection step. These complementary approaches will allow understanding of the complex fungal sulfur pathway. Long term goal is to establish the role of the sulfur network during the interaction between the plant and the fungus.

P4–33

Transcriptional responses of *Pinus sylvestris* following challenge with a saprotrophic, symbiotic or pathogenic fungus at post penetration interaction stages.

Gregory Heller[1], Guosheng Li[1], Jason Osborne[2], Deborah Craig[3], Leonel van Zyl[3], Ron Sederoff[3], Ralph A. Dean[4], Roger Finlay[1], Jan Stenlid[1], Frederick O. Asiegbu[1].

[1]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala Sweden. [2]Dept of Statistics, North Carolina State University (NCSU), Raleigh USA. [3]Forest Biotechnology Laboratory, NCSU, Raleigh USA. [4]Fungal Genomics Laboratory, NCSU Raleigh, USA.

Gregory.Heller@mykopat.slu.se

Analyses of specificity in host responses at late stages (15dpi) of interactions of *Pinus sylvestris* with a saprotroph (*Trichoderma aureoviride*), a pathogen (*Heterobasidion annosum*) or a symbiotic ectomycorrhizal fungus (*Laccaria bicolor*) were conducted. The mRNA profiling technology was used to identify genes that are either induced or suppressed during the interaction. Micro-array containing 2176 individual cDNA fragments representing a diverse range of transcripts expressed in conifer trees during different levels of development were examined. The gene expression data from the cDNA micro-array were analysed using the 2-interconnected mixed linear model statistical approach. A total of 421 differentially expressed genes were identified with 371, 36 and 14 genes specifically up-regulated in response to pathogenic, mycorrhiza and saprotrophic interactions respectively. After 15 days, unique and distinct expression profiles for each interaction combination was documented. The results were further compared with data obtained for expression profiles at early stages of the interaction (1, 5dpi). There was no specificity in response of conifer trees to presence of the various fungi at 1 dpi. Only at later stages of interaction (5, 15dpi) specific distinctions and recognitions of the fungi by the tree were observed. The general trend is that the number of up-regulated genes increased over time in pathogenic interactions whereas the converse was the case for mycorrhiza and saprotrophic interactions. The data will further be validated by Real Time PCR and the overall results will allow for a better understanding of molecular basis for specificity in recognition of conifer trees to diverse microbial species.

P4–34

Use of a heterologous cDNA array for transcriptome profiling of interaction events in *Pinus sylvestris* following challenge with a pathogenic, saprophytic or symbiotic fungus.

Aleksandra Adomas[1], Tzu-Ming Chu[3], Deborah Craig[2], Len van Zyl[2], Russ Wolfinger[3], Ron Sederoff[2], Ralph A. Dean[4], Roger Finlay[1], Jan Stenlid[1], Frederick O. Asiegbu[1].

[1]Department of Forest Mycology and Pathology, Swedish University of Agriculture, Uppsala Sweden. [2]Forest Biotechnology Laboratory, North Carolina State University, Raleigh USA. [3]SAS Institute, Raleigh, NC USA. [4]Fungal Genomics Laboratory, North Carolina State University, Raleigh, USA.

Aleksandra.Adomas@mykopat.slu.se

The response mechanisms, recognition and specificity of conifer trees during interaction with pathogenic or non-pathogenic fungi were investigated. The roots of *Pinus sylvestris* were challenged for five days with a pathogenic (*Heterobasidion annosum*), saprotrophic (*Trichorderma aureoviride*) or symbiotic ectomycorrhizal (*Laccaria bicolor*) fungus. The gene expression data from cDNA micro-arrays consisting of 2176 *Pinus taeda* genes were analysed using 2-interconnected mixed linear model statistical approach. The result of the pairwise comparisons of the different treatments against un-inoculated control led to identification of a total of 266, 110 and 180 up-regulated and 316, 119 and 411 down regulated genes in the pathogenic, saprotrophic and mycorrhiza interactions respectively. The results were compared with similar data obtained for pre (1dpi) and late (15 dpi) colonization interaction stages. The total number of genes differentially expressed in the pathogenic interaction five days after infection (5 dpi) was higher by 70% than on the early interaction stage (1 dpi). In the non-pathogenic interactions an average decrease of 47% of genes differentially expressed was observed. Hierarchical clustering of the differentially expressed transcripts showed a specific pattern for pathogenic, saprotrophic and mycorrhiza interactions at 5 dpi compared to the pre-colonisation stage. Further analysis of the clustered genes indicated that they encode several diverse classes of proteins. Real Time PCR analysis is currently in progress to validate the data. The result of this comprehensive expression profiling will hopefully shade more light on the mechanistic basis for recognition and response of conifer trees to pathogenic and non-pathogenic fungi.

P4–35

Comparative analyses of resistant and susceptible *Theobroma cacao-Crinipellis perniciosa* interactions using ESTs.

Abelmon Gesteira[1], Aline da Silva[1], Fabienne Micheli[1,2], Robson José Costa[1], Nicolas Carels[3], Joci Neuby Alves Macêdo[1], Geruza de Oliveira Ceita[1], Karina Peres Gramacho[4], Diego Suárez Frias[3] and Júlio Cézar de Mattos Cascardo[1].

[1]Laboratório de Genômica e Expressão Gênica, UESC, Ilhéus-BA, Brasil. [2]Cirad-CP, UMR PIA, Montpellier, France. [3]LABBI, UESC, Ilhéus-BA, Brasil. [4]Laboratório de Fitopatologia Molecular, CEPEC, Ilhéus-BA, Brasil.

abelmon@uesc.br

Since 1989, the Witches' broom disease due to the pathogenic fungus *Crinipellis perniciosa* increased in Bahia, Brazil, destroying the cultivation of cacao trees and leading to important economical and social changes in the concerned areas. The aim of the research developed in the laboratory is to acquire a good knowledge of the determinism of the interaction between the cacao tree and the pathogen *Crinipellis* based on functional genomic studies, and to contribute to the development of effective methods for the genetic improvement of resistance to this disease, so as to create new varieties obtained through a Marker-Assisted Selection (MAS) strategy.

We report here studies related to gene expression in meristems of resistant and susceptible cacao trees inoculated or not with *C. perniciosa* spores. Plants were harvested from time zero up to 70 days after inoculation (necrosis symptoms), and used to generate Expressed Sequence Tags (ESTs). First, we developed a successful and reliable procedure for the isolation of RNA from any cacao tissues particularly those containing a high content of polyphenols and polysaccharides such as meristems and fruits. Then, we generated two cDNA libraries corresponding to inoculated resistant and susceptible plants, and we randomly sequenced and analyzed using bioinformatics tools, 5 000 ESTs of both

libraries. Analysis of EST cluster distribution, supported by array experiments enabled us to identify a large number of novel genes predicted up- or down-regulated during the compatible or resistant cacao-*Crinipellis* interaction. Interestingly, the sequences obtained from susceptible plants are closely related to genes involved in program cell death.

In a short term we plan to do functional analyses of some candidate genes, which can be used for mapping, to facilitate introgression strategies and to create new varieties resistant to *Crinipellis perniciosa*. These data may also be related to research on gene expression of cacao challenged with other pathogens, such as *Phytophthora ssp*.

P4–36

Gene expression during switch from saprotrophic to pathogenic phases of growth in the root and butt rot fungi *Heterobasidion annosum*.

Karl Lundén[1], Fred Asiegbu[1].
[1]*Dept. Of Forest Mycology and Pathology, SLU, Sweden.*
Karl.Lunden@mykopat.slu.se

The tree pathogen *Heterobasidion annosum* can prevail in dead roots and from there spread to living trees, therefore we examined if a shift in gene expression occurred during the switch from saprotrophic to pathogenic stage. We used a macro-array differential gene analysis to identify genes that are either induced or suppressed during either stages of growth of the fungus. Macro-array's containing a selected number of clones from cDNA library of *H.annosum* and *H. parviporum* representing functionally diverse range of genes were investigated. Dead pine seedlings were inoculated with *H. annosum* and transferred to water agar plates containing living pine seedlings, the hyphae were then sampled from various stages of interaction before and after contact with the pine host. Total RNA will be isolated, reverse transcribed into cDNA to be used as probes for differential screening of the macro-array membranes. Signal intensity values for differentially expressed genes will be documented with Quantity one (Bio-RAD) and the data will be statistically analysed to identify significantly up and down-regulated genes.

P4–37

Genes up-regulated in a tolerant Cavendish banana in response to *Fusarium oxysporum* f. sp. *cubense* infection.

Noëlani van den Berg[1], Ingo Hein[2], Paul Birch[2], Altus Vijoen[1], Dave Berger[3].
[1]*Dept. Micobiol. & Plant Pathol.,* [3]*Dept. Botany, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa,* [2]*Scottish Crops Research Institute, Invergowrie, Dundee, Scotland.*
Noelani.vdberg@fabi.up.ac.za

Fusarium oxysporum f. sp. *cubense* (*Foc*), a soil-borne pathogen of banana, has been responsible for one of the most devastating disease epidemics in agricultural history. The only control strategy has been to replace susceptible varieties such as Gros Michel with tolerant Cavendish bananas. However, Cavendish bananas are highly susceptible to *Foc* race 4. The aim of this study was to identify differentially expressed genes in a tolerant Cavendish selection (DRS1) when compared to susceptible plants (Williams), after challenge with *Foc* in the greenhouse. A cDNA library enriched for genes up-regulated in DRS1 after *Foc* infection was constructed utilising suppression subtractive hybridisation (SSH). The library was screened using DNA microarray technology[a] and 334 potentially induced clones were selected and sequenced. Several defence related transcripts were identified by BLASTX searches, including: PR1, pectin acetylesterase, xylanase inhibitor, peroxidase, catalase 2, metallothionein, isoflavone reductase and tripsin inhibitor. Inverse Northern dot blot data indicated that all of the above-mentioned transcripts were up-regulated 6 hours post inoculation (hpi) in DRS1 compared to Williams. Real Time RT-PCR results confirmed that pectin acetylesterase, PR1 and catalase 2 were expressed at higher levels in DRS1 compared to Williams. PR1 and catalase 2 showed increased expression over time, while pectin acetylesterase showed a significant increase in expression at 3 hpi. Further sequencing and analyses of gene expression and function will be performed to advance our understanding of *Foc* tolerance in DRS1.

[a]Van den Berg *et al.* 2004, *BioTechnique* 37(5): 818-824.

P4–38

Suppression of *BvPAL* transcription after pathogen attack occurs at the core promoter.

Klaus Schmidt[1], Brigitte Heberle[2], Jeanette Kurrasch[1], Reinhard Nehls[1] and Dietmar J. Stahl[1].
[1]*PLANTA Angewandte Pflanzengenetik und Biotechnologie GmbH, Einbeck, Germany;* [2]*KWS Saat AG, Einbeck, Germany.*
K.Schmidt@KWS.de

Fungal suppressors of plant defence reactions play a crucial role in causing plant diseases. The structure and the mode of action of the suppressors are largely unknown. In the early phase of infection of sugar beet (*Beta vulgaris* L.) leaves with the phytopathogenic fungus *Cercospora beticola*, we observed a reduction of the expression of the phenylalanine ammonia lyase (*BvPAL*) and cinnamic acid 4-hydroxylase (*BvC4H*) genes. *BvPAL* reduction was found at the transcript and at the enzyme activity level. The region within the *BvPAL*-promoter required for the reduction of transcription was identified by transient biolistic experiments to the position –34 to +45, relative to the transcriptional start site. This region is equivalent to the minimal promoter, surrounding the TATA-Box and the transcriptional start site.

P4–39

Functional Characterisation of a Cellulose Synthase from *Phytophthora infestans* using Proteomics and RNA interference.

Laura J. Grenville-Briggs[1], Catherine R. Bruce[1], Anna O. Avrova[2], Stephen C. Whisson[2], Alison Williams[1], Vicky L. Anderson[1], Paul R.J. Birch[2] and Pieter van West[1].
[1]*Aberdeen Oomycete Group, University of Aberdeen, IMS, Foresterhill, Aberdeen, AB25 2ZD, UK.* [2]*Plant-Pathogen Interactions Programme, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK.*
l.grenville-briggs@abdn.ac.uk

A thorough understanding of the molecular events taking place during early interactions between *P. infestans* and host and non-host plants is crucial for developing new control measures. The appressorial stage of the interaction is one of the first moments in which direct contact between the pathogen and the plant occurs via the formation of highly specialised infection structures, including the appressorium, penetration peg, and the infection vesicle. Moreover, it is during this phase that plant defence responses are initiated.

We have isolated proteins that are abundant in appressoria using proteomics, one of which is a cellulose synthase (*Pi-Cel1*). Characterisation of the gene encoding this protein reveals both cellulose synthase and glycosyl-transferase signatures, and uniquely, two pleckstrin (PH) domains. PH domains are found in eukaryotic signalling molecules, and may facilitate anchoring to membranes. They have also been shown to interact with lipid molecules and Gß subunits. The role of the PH domain in Pi-Cel1 is as yet unknown. Two other Cellulose synthase genes, *Pi-Cel2, Pi-Cel3,* have been identified within *P. infestans* EST collections. Real Time RT-PCR reveals that all three genes are up-regulated in germinating cysts with appressoria, relative to the mycelial state. Gene silencing studies employing RNA interference of *Pi-Cel1* perturbs the morphology and structure of appressoria. Here we discuss our latest results.