

able to infect unwounded, mature reed leaves. Furthermore, 30 true fungi have been detected by conventional isolation methods and 350 different fungi by molecular methods. The distribution of these organisms in reed organs is highly variable and only few fungi dominate in each organ. Some *Stagonospora* spp. show mutualistic capabilities towards the vitality of reed resulting in increased growth. Moreover, some fungal species exhibit antagonistic effects against the reed pathogen *P. phragmitis*. PCR based methods have been developed to detect and identify beneficial and pathogenic organisms in reed. For the molecular detection of antagonists and pathogens a PCR system based on species specific primer pairs localised in the ITS-region of the rDNA has been established. In addition, a macroarray for the analysis of niche differentiation of the most abundant and antagonistic true fungi, as well as the most abundant and pathogenic oomycetes in reed plants has been designed. The methods described provide an appropriate means to exhaustively investigate and characterise the mycoflora associated with stands of common reed.

### PS 3-247

#### ANALYSIS OF THE SECRETOME OF *ARABIDOPSIS* INFECTED WITH THE OOMYCETE *HYALOPERONOSPORA PARASITICA* BY SEMI-QUANTITATIVE MASS SPECTROMETRY

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The intercellular space, or apoplast, plays an important role in the interaction between plants and microbes. Processes like nutrient exchange and defense by extracellular proteins and compounds take place in the apoplast. Upon infection by a pathogen plant defense responses are elicited resulting in secretion of a wide array of defense-related proteins, or pathogenesis-related (PR)-proteins. To establish a successful infection a pathogen needs to overcome these defense-responses and manipulate host-cell metabolism and transport to obtain nutrients. To get insight into the changes in the apoplast proteome during the infection process, intercellular washing fluids (IWFs) of a compatible interaction between *Arabidopsis* accession *Ler* infected with *H. parasitica* isolate Cala2 were isolated. IWFs were subjected to semi-quantitative analysis by SDS-PAGE and LC-MS-MS. Over a 100 putatively secreted proteins were identified in two independent experiments, of which 44 were differentially present compared to IWFs from mock-inoculated plants. Examples of differentially present defense-related proteins are peroxidases, chitinases and glycosyl hydrolases. An important second class of proteins could be assigned to the downy mildew pathogen. Identification was achieved using the *H. parasitica* genome assembly draft 2.0 and in-house EST data. We will report on the functional studies that have been initiated to study the potential role of secreted host and pathogen proteins in disease susceptibility.

### PS 3-248

#### DEFINING MOLECULAR RECOGNITION OF *ALBUGO CANDIDA* BY ITS HOST PLANT.

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White rust caused by the obligate oomycete *Albugo candida* (Pers. ex. Fr.) O. Kuntze affects both crop and wild Brassicaceae species including cultivated Brassica crops and Arabidopsis. In order to understand the mechanisms of plant defence regulation it is important to identify the key plant and pathogen molecules (so called pathogen effectors) involved in the interaction of plant and pathogen. We used Arabidopsis to study the interaction with two isolates of *A. candida*, Ac2v and Ac7v from *Brassica juncea* and *B. rapa* respectively. Assessment of the natural variation found among Arabidopsis ecotypes, revealed that Columbia (Col) exhibited a resistant and both Niederzenz (Nd) and Wassilewskija (Ws) exhibited a susceptible phenotype when challenged with either Ac2v or Ac7v. The resistance in Col was conferred by a single locus named *RAC4* (resistance to *A. candida*) encoding a 113.9 KDa TIR-NB-LRR type protein. To identify *A. candida* effector molecules that regulate the *RAC4* initiated defence response, cDNA sequencing and cDNA-AFLP were employed. A cDNA library was constructed using *B. juncea* cultivar 'Cutlass' infected with the compatible isolate Ac2v. Approximately, 14,000 clones were sequenced and compared with plant and pathogen sequences in public databases as well as the Brassica EST databases developed at Saskatoon Research Centre. This analysis revealed that 10,300 of the ESTs were of plant origin and 3,500 were potentially from *A. candida* Ac2. Of the 3,500 *Albugo* ESTs, four ESTs contained the RXLR motif. mRNA was isolated from 'Cutlass' for cDNA-AFLP analysis to compare infected and non-infected tissues at 2, 4 and 8 days after inoculation with Ac2v. Pathogen genes that were expressed at the early stages of plant infection were identified using cDNA-AFLP. Additional information about the candidate effector molecules identified from the *Albugo* EST collection, will be obtained by isolating and analyzing their corresponding genomic clones from an Ac2v BAC library.

### PS 3-249

#### *ARABIDOPSIS* EXPRESSING THE *PHYTOPHTHORA INFESTANS* EFFECTOR GENE *IPIO* SHOWS ALTERED DISEASE SUSCEPTIBILITY

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*Phytophthora infestans* secretes numerous effectors that could play a role in colonizing host plants. One of these is IPI-O, a protein that belongs to a large class of highly divergent effectors sharing a short conserved motif called RxLR-dEER. The *ipiO* gene is not expressed in mycelium but highly expressed in infected leaves, specifically in the periphery of water-soaked lesions and in healthy looking tissue surrounding the lesion (Van