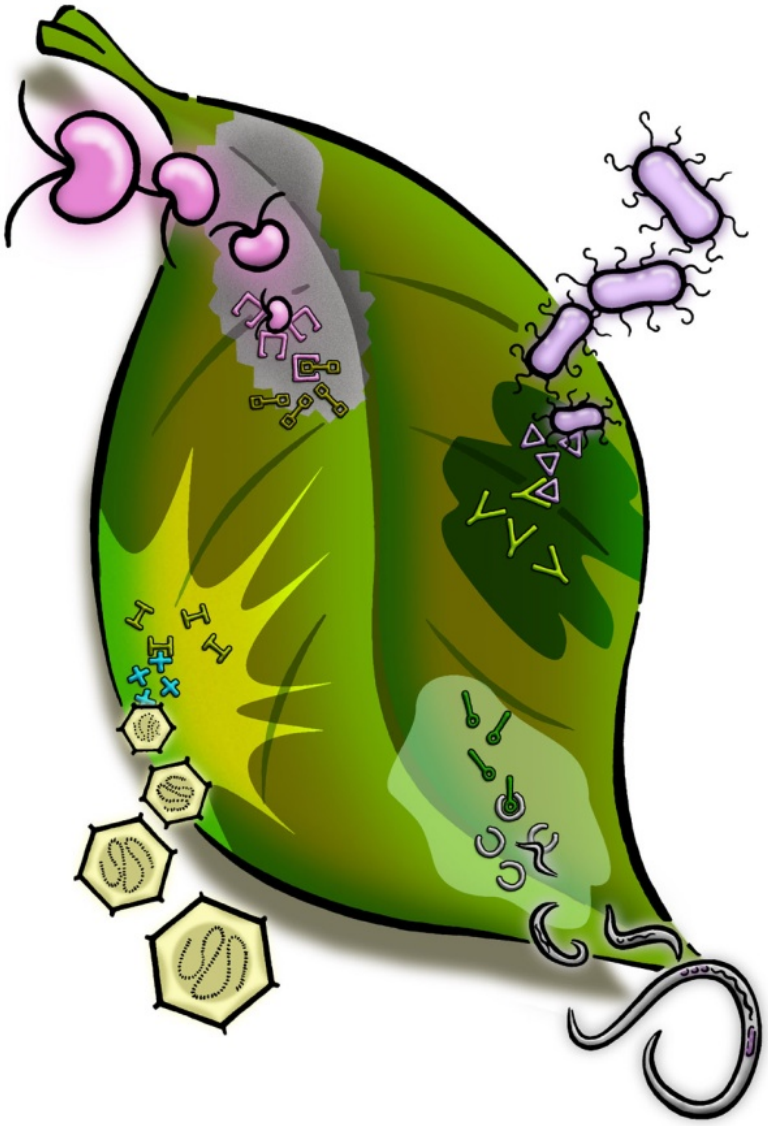
Small secreted protein lost in large A+T-rich isochores as effectors in Leptosphaeria maculans:the case study of LmCys2

Isabelle Fudal-Grolier Fudal, Bronislava Profotova, Francoise F. Blaise, Marie-Helene Balesdent, Thierry T. Rouxel

22nd New Phytologist Symposium

Effectors in plant–microbe interactions

INRA Versailles Research Centre, Paris, France
13–16 September, 2009



Programme, abstracts and participants

New Phytologist

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Effectors in plant–microbe interactions

INRA Versailles Research Centre, Paris, France

Organizing committee

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Programme, abstracts and participant list compiled by Jill Brooke.
'Effectors in plant-microbe interactions' illustration by A.P.P.S., Lancaster, U.K.

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Programme

Sunday 13 September (Hotel Novotel Château de Versailles)

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Monday 14 September (INRA Versailles)

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Session 1:	**Genome-wide analyses of microbial effectors** ***Chair: Marc-Henri Lebrun***
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09:15–09:55	**1.2 *Ralstonia solanacearum*: Molecular basis of adaptation to plants** *Stéphane Genin*
Session 2:	**Effector evolution** ***Chair: Marc-Henri Lebrun***
09:55–10:35	**2.1 The evolution of the *Pseudomonas syringae* HopZ family of type III effectors** *David Guttman*
10:35–11:15	**2.2 Evolutionary and functional dynamics of *Phytophthora infestans* effector genes** *Sophien Kamoun*
11:15–11:45	**Coffee**
Session 3:	**Microbial effector functions: virulence and avirulence** ***Chair: Nick Talbot***
11:45–12:25	**3.1 Elicitation and evasion of plant immunity by *Pseudomonas* effectors AvrPto and AvrPtoB** *Greg Martin*
12:25–13:05	**3.2 *Pseudomonas syringae* type III effectors: Enzymatic activities, sites of action, and their ability to suppress plant innate immunity** *Jim Alfano*
13:05–14:00	**Lunch**
14:00–14:40	**3.3 Flax rust Avr-R interactions** *Peter Dodds*
14:40–15:20	**3.4 *Leptosphaeria maculans* AVRs and SSPs** *Thierry Rouxel*

15:20–16:00	**3.5 The pathogen effectors of the downy mildew oomycete pathogen, *H. arabidopsidis,* and host responses to stress** *Jim Benyon*
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16:30–17:10	**3.6 *Cladosporium fulvum* effectors and functional homologues in Dothideomycete fungi** *Pierre de Wit*
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09:10–09:50	**4.2 Host-selective toxins of *Pyrenophora tritici-repentis*, inside and out** *Lynda Ciuffetti*
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11:00–11:40	**5.2 Bacterial effector delivery** *Guy Cornelis*
11:40–12:20	**5.3 How oomycete and fungal effectors enter host cells** *Brett Tyler*
Session 6:	**Plant targets of microbes/bioagressor effectors** ***Chair: Nick Talbot***
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14:15	**Afternoon excursion to the Palace of Versailles**
18:00	**Bus leaves Hotel Novotel Château de Versailles for Conference Dinner with a cruise on the River Seine**

Wednesday 16 September	
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09:10–09:50	**6.3 Small RNA pathways and their interference by pathogens in eukaryotes** *Olivier Voinnet*
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Session 8:	**Emerging Effectors – nematodes, insects, metabolites** ***Chair: Sophien Kamoun***
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16:30–17:10	**8.4 Secondary metabolites as effectors: Fungal secondary metabolism is an essential component of the complex interplay between rice and *Magnaporthe grisea*** *Marc-Henri Lebrun*
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17:30	**Meeting close and depart**

Speaker Abstracts

Session 1: Genome-wide analyses of microbial effectors
Chair: Marc-Henri Lebrun

1.1 *Ustilago* effectors

REGINE KAHMANN, K. SCHIPPER, A. DJAMEI, T. BREFORT, G. DOEHLEMANN, F. RABE, J. WU, L. LIANG, G. BAKKEREN*, J. SCHIRAWSKI
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The basidiomycete fungus *Ustilago maydis* is a biotrophic maize pathogen that codes for a large set of novel secreted effector proteins. A significant percentage of the respective genes is clustered in the genome and upregulated during pathogenic development (Kaemper *et al*., 2006). Many of these gene clusters have crucial roles during discrete stages of biotrophic growth. We now show that *U. maydis* is eliciting distinct defense responses when individual clusters or individual genes, respectively, are deleted. Maize gene expression profiling has allowed us to classify these defense responses and provides leads to where the fungal effectors might interfere. We describe where the crucial secreted effector molecules localize, their interaction partners and speculate how this may suppress the observed plant responses. A comparative genomics approach in which the genomes of the related smut fungi *Sporisorium reilianum*, *U. scitaminea* and *U. hordei* were sequenced using 454-technology has revealed that these genomes contain paralogs of most effectors found in *U. maydis*. However, resulting from a coevolutionary arms race between pathogen and host these effectors are highly divergent compared to other proteins. This aids their detection and functional analysis.

1.2 *Ralstonia solanacearum*: Molecular basis of adaptation to plants

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Ralstonia solanacearum is a devastating plant pathogen with wide geographic distribution and an unusually wide host range since it is the agent of vascular wilt disease in more than 200 plant species. Host range is directly controlled in some cases by Type III effectors, either by extending or restricting the ability of *R. solanacearum* to infect and multiply on given hosts. Comparative genomics can provide insights about the evolution of some avirulence gene sequences that could result in a better adaptability of the pathogen. An experimental evolution approach by maintaining the bacterium on fixed plant lines by serial passage experiments for over 300 generations and aimed to identify the genetic basis of the adaptation of the bacterium to different host plants will also be presented.

Session 2: Effector evolution
Chair: Marc-Henri Lebrun

2.1 The evolution of the *Pseudomonas syringae* HopZ family of type III effectors

DAVID S. GUTTMAN, JENNIFER D. LEWIS, AMY H. LEE, PAULINE W. WANG, YUNCHEN GONG, DARRELL DESVEAUX
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The plant pathogen *Pseudomonas syringae* uses the type III secretion system to secrete and translocate effector proteins into its plant hosts. Many of these effectors suppress host defense signaling and / or induce resistance (R) protein-mediated defenses. The YopJ / HopZ family of effectors is a common and widely distributed class found in both animal and plant pathogenic bacteria. In previous work, we showed that the *P. syringae* HopZ family includes three major allele types (one ancestral and two brought in by horizontal gene transfer) whose diversification was driven by the host defense response (Ma *et al.,* 2006), and that virulence and defense induction phenotypes are strongly allele-specific (Lewis *et al.,* 2008). We have now the R protein responsible for HopZ1a-dependent immunity. This previously uncharacterized R protein functions independently of RIN4 and all other known R proteins, and shows HopZ effector allele specificity. Further, we designed a novel, high-throughput interactor screen using next-generation genomics technology to elucidate the HopZ-R protein resistance complex and host targets of all the HopZ alleles. This diverse effector family beautifully illustrates how natural genetic variation modulates host target and R protein specificity and influences host specific virulence and defense.

2.2 Evolutionary and functional dynamics of *Phytophthora infestans* effector genes

SOPHIEN KAMOUN
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It is now well established that oomycete plant pathogens secrete effectors that target the apoplast or are translocated inside host plant cells to enable parasitic infection. Apoplastic effectors include several types of inhibitor proteins that interfere with the activities of extracellular plant hydrolases. Host-translocated (cytoplasmic) effectors include the RXLR and Crinkler (CRN) families, which carry conserved motifs that are located downstream of the signal peptide and mediate delivery into host cells. How these effectors perturb plant processes remains poorly understood although some RXLR effectors are known to suppress plant immunity. This presentation will report on the progress we made in unravelling the evolutionary dynamics of effector genes of the potato late blight pathogen *Phytophthora infestans*. More specifically, we will focus on the insights obtained from sequencing the genomes of *P. infestans* and that of four closely related species, and our progress in deciphering the virulence activities of *P. infestans* effectors.

Session 3: Microbial effector functions: virulence and avirulence
Chair: Nick Talbot

3.1 Elicitation and evasion of plant immunity by *Pseudomonas* type III effectors AvrPto and AvrPtoB

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Pseudomonas syringae pv. *tomato*, which causes bacterial speck disease of tomato, delivers ~30 type III effector proteins into the host cell. Tomato varieties that are resistant to speck disease express the Pto kinase which physically interacts with two of these effectors, AvrPto or AvrPtoB, and activates a variety of host immune responses including localized programmed cell death. The Pto kinase is encoded by one member of a clustered, five-member gene family. Another member of this family, Fen, recognizes certain truncated versions of AvrPtoB. An NBARC-LRR protein, Prf, is required for Pto- and Fen-mediated immunity.
AvrPto (18 kD) and AvrPtoB (60 kD) both make significant contributions to *P. syringae* virulence and have many other similarities. Each is a modular protein with discrete domains that have distinct activities. One of these domains in both proteins targets the FLS2/BAK1 complex to disrupt PAMP-triggered immunity. In each effector, this domain forms a contact surface involved in the interaction with Pto. Both effectors have an additional unique contact with Pto and their structures overall are very different. Host-mediated phosphorylation of each effector promotes its virulence activity and for AvrPto this phosphorylation-dependent virulence activity was found to be independent of the FLS2/BAK1 disruption mechanism. Interestingly, each effector is targeted by two different resistance proteins that recognize a structural element important for effector virulence activity. I will present recent data regarding the molecular basis of the multiple virulence activities of AvrPto and AvrPtoB. Supported by NIH-R01GM078021 and NSF-DBI-0605059.

3.2 *Pseudomonas syringae* type III effectors: Enzymatic activities, sites of action, and their ability to suppress plant innate immunity

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The bacterial pathogen *Pseudomonas syringae* is dependent on a type III protein secretion system and the type III effector proteins (T3Es) it injects into host cells to cause disease. The enzymatic activities of T3Es and their plant targets remain largely unknown. I will discuss our progress on the DC3000 T3E HopU1, which we determined is a mono-ADP-ribosyltransferase (ADP-RT). Using ADP-RT assays coupled with mass spectrometry we identified the major HopU1 substrates in *Arabidopsis thaliana* extracts to be several RNA-binding proteins that possess RNA-recognition motifs (RRMs). HopU1 ADP-ribosylates an arginine residue in position 49 of the glycine-rich RNA-binding protein *At*GRP7, which is within its RRM. We found that ADP-ribosylated *At*GRP7 was reduced in its ability to bind RNA. Another T3E that we are currently focused on is HopG1, which localizes to plant mitochondria and when expressed transgenically result in plants that are infertile, dwarfed, and possess increased branching. HopG1 also has the ability to suppress innate immunity, which suggests that pathogens may target mitochondria as a pathogenic strategy. Finally, I will also discuss recent experiments that suggest that the majority of DC3000 type III effectors can suppress plant immunity.

3.3 Flax rust Avr-R interactions

PETER DODDS[1], M. RAFIQI[2], P. H. P. GAN[2], M. BERNOUX[1], M. RAVENSDALE[1], M. KOECK[1], G. LAWRENCE[1], B. KOBE[3], D. A. JONES[2] A. R. HARDHAM[2], J. ELLIS[1]
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Flax rust (*Melampsora lini*) is a biotrophic basidiomycete pathogen that infects flax plants (*Linum usitatissimum*). Nineteen different rust resistance genes have been cloned from flax, including 11 allelic variants of the *L* locus, which all encode cytosolic TIR-NBS-LRR proteins. Four families of Avr genes, *AvrL567*, *AvrM*, *AvrP123* and *AvrP4* have been identified in flax rust and all encode small secreted proteins that are expressed in haustoria. Recognition occurs inside the plant cell and yeast-two-hybrid analyses indicate that, in at least two cases, this is based on direct interaction with the corresponding cytosolic NB-LRR R proteins. This suggests that the Avr proteins are translocated into host cells during infection, and immunolocalisation experiments have detected the AvrM inside host cells during infection. Expression of various GFP-tagged AvrL567 and AvrM mutants in plants suggest that these proteins are taken up into host cells in the absence of the pathogen and that this transport is dependent on sequences in the N terminal region. Although the LRR domain is primarily responsible for determining recognition specificity of the flax R proteins, y2h assays indicate that a functional NBS domain is also required for Avr protein interaction. The TIR domain is not required for recognition and several TIR mutations disrupt HR induction, without affecting recognition. Furthermore, overexpression of the TIR domain alone induces HR, suggesting a primary signalling role for this domain. Direct recognition has led to strong diversifying selection in the rust Avr genes to escape recognition and host resistance.

3.4 *Leptosphaeria maculans* AVRs and SSPs

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The genome of the ascomycete *Leptosphaeria maculans* shows the unusual characteristics to be organized in isochores, i.e., the alternating of homogeneous GC% regions with abrupt changes from one to the other. GC-equilibrated isochores (average 52% GC) are gene-rich whereas AT-rich isochores (40–43% GC) are mostly devoid of active sequences and are made up of mosaics of intermingled and degenerated repeated elements. The three avirulence (*AvrLm*) genes identified so far in this species are “lost in middle of nowhere” genes, isolated in the middle of large AT-rich isochores. Our postulate thus was that AT-rich isochores were specific “ecological niches” for avirulence genes and effectors in *L. maculans*. This was firstly validated by analysis of three genes lying in the same genome environment (*LmCys* genes) and showing the same characteristics as *AvrLm* genes (low GC content, strong overexpression at the onset of plant infection, encoding for small secreted proteins -SSP- often rich in cysteines). Of these, one, *LmCys2*, was shown to act as an effector (see I. Fudal *et al*. poster). A systematic search for SSP as effector candidates was performed using bioinformatics. 455 AT-rich isochores were extracted from the genome data and their repeat content masked using the *L. maculans* repeated element database. Non-repeated regions were then investigated with a pipe-line dedicated to the identification of SSP. This provided us with three datasets: 529 SSP-encoding genes in GC-equilibrated isochores, 498 non-SSP- and 122 SSP-encoding genes in AT-rich isochores. Part of this latter set of genes was analyzed for their occurrence in natural populations and expression data *in vitro* and *in planta*. Finally, the 122 putative AT-SSP showed structural features reminiscent of the *AvrLm* and *LmCys* genes and occasional RxLR-like motifs. Possible diversification mechanisms favoured by this genome location will be discussed.

3.5 The pathogen effectors of the downy mildew oomycete pathogen, *H. arabidopsidis,* and host responses to stress

JIM L. BENYON
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To enable a pathogenic lifestyle many organisms produce a repertoire of proteins that enable them to colonize host tissue. These proteins, effectors, are likely to be targeted to suppressing host immune mechanisms and redirecting nutritional resources to benefit the pathogen. We are studying the interaction between the downy mildew pathogen *Hyaloperonospora arabidopsidis* and Arabidopsis. In a community collaborative effort we have just completed the sequencing and annotation of the *H. arabidopsidis* genome and it reveals a gene content that suggests that it has been adapted to a biotophic lifestyle. It has a very large effector content that suggests complex mechanisms of interaction between host and pathogen. We are analyzing the role of individual effector proteins in interacting with the host plant immune system via yeast two hybrid analyses. Finally, we are analyzing the role nature of the host plant response to biotic and abiotic stress using systems biology approaches.

3.6 *Cladosporium fulvum* effectors and functional homologues in Dothideomycete fungi

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Cladosporium fulvum is a biotrophic pathogen that causes leaf mould of tomato. So far, ten effectors have been identified from this fungus including avirulence (Avrs: Avr2, Avr4, Avr4E and Avr9) and extracellular proteins (Ecps: Ecp1, Ecp2, Ecp4, Ecp5, Ecp6 and Ecp7). All Avrs and Ecps are assumed to be virulence factors. Avr2 is an inhibitor of apoplastic plant cysteine proteases and Avr4 is a chitin-binding protein that protects chitin present in the cell walls of fungi against deleterious effects of plant chitinases during infection. Ecp6 contains chitin-binding LysM domains that are supposed to bind chitin fragments released in the apoplast during infection. Recently we have sequenced the genome of race 0 of *C. fulvum* that enabled us to perform initial comparative genome analyses with other sequenced members of the plant pathogenic Dothideomycetes. So far, the genome of *C. fulvum* is most related to *Mycosphaerella fijiensis* the causal agent of black Sigatoka, a devastating fungal disease of banana. We have identified functional homologues of *C. fulvum* Avr4, Ecp2 and Ecp6 effectors in Dothideomycetes, including *M. fijiensis, M. graminicola, Cercospora nicotianae* and *C. beticola.* We have also shown that Avr4 and Ecp2 are not only structural but also functional homologues of the *C. fulvum* effectors.

3.7 How *Xanthomonas* type III effector proteins manipulate the plant

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We study the interaction between pepper and tomato and the Gram-negative bacterium *Xanthomonas campestris pv.vesicatoria* (*Xcv*), which causes bacterial spot disease on pepper and tomato. Successful interactions of *Xcv* with the plant depend on the type III secretion (T3S) system, a molecular syringe which injects 20-30 effector proteins (termed Avr or Xop = Xanthomonas outer protein) into the plant cell cytoplasm. One of the best understood type III effectors is AvrBs3, which functions as transcription factor in the plant cell nucleus and affects both susceptible and resistant plants. *Xcv* strains expressing AvrBs3 induce the hypersensitive reaction (HR; programmed cell death) in pepper plants carrying the resistance gene *Bs3*. In pepper plants lacking *Bs3* and other solanaceous plants AvrBs3 induces a hypertrophy (cell enlargement) of mesophyll cells that probably helps to disseminate the bacteria. AvrBs3 activity depends on a central region of tandem repeats, its localization to the plant cell nucleus and the presence of an acidic activation domain. One of the direct targets of AvrBs3 is *UPA20* (*UPA*, upregulated by AvrBs3) which encodes a transcription factor and is a key regulator of hypertrophy. New insights into AvrBs3 action will be presented.

Session 4: Effector trafficking: processing/uptake by plants
Chair: Francis Martin

4.1 Effector secretion and translocation during rice blast disease

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To cause disease, *Magnaporthe oryzae* sequentially invades living rice cells using specialized intracellular invasive hyphae (IH) that are enclosed in host-derived Extra-Invasive-Hyphal Membrane. Blast IH specifically express numerous Biotrophy-Associated-Secreted (BAS) proteins including known effectors, AVR-Pita, PWL1, and PWL2. We identified a highly-localized pathogen-induced structure, the Biotrophic Interfacial Complex (BIC), which accumulates fluorescently-labeled effectors and other BAS proteins secreted by IH. BICs contain complex lamellar membranes, and are associated with dynamically-shifting host cytoplasm. In successively invaded rice cells, fluorescent effectors were first secreted into BICs at the tips of filamentous hyphae that entered the cell. Fluorescent BICs then moved off the hyphal tips and remained beside the first differentiated IH cells as IH continued their colonization. Fluorescent effectors that accumulated in BICs were translocated to the cytoplasm of invaded host cells. Translocated effectors were also observed in uninvaded neighboring cells, suggesting that the fungus sends effectors to hijack host cells before entry.

4.2 Host-selective toxins of *Pyrenophora tritici-repentis*, inside and out

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Host-selective toxins (HSTs) are virulence factors produced by plant pathogenic fungi. Often, host-selective toxins follow an inverse gene-for-gene interaction where a single locus in the host is responsible for toxin sensitivity. The ability of these virulence factors to promote cell death by a variety of mechanisms benefits the necrotrophic life style of the fungus. Our long-term goal is to fully describe the molecular interactions of the host-selective toxin producing fungus, *Pyrenophora tritici-repentis*, with its host plant, wheat. This includes the identification and characterization of genes involved in pathogenicity and host specificity, the mechanisms by which this fungus acquires these virulence factors, and the determination of the molecular site- and mode-of-action of these toxins. ToxA and ToxB are two proteinaceous HSTs of *P. tritici-repentis* which promote virulence through distinctly different mechanisms. ToxA induced changes occur rapidly and result in necrosis. In contrast, the plant responses to ToxB are slower and result in chlorosis. High affinity binding to a plant receptor and rapid internalization of ToxA leads to altered Photosystem homeostasis, the accumulation of reactive oxygen species and major transcriptional reprogramming. Unlike ToxA, ToxB appears to have an extracellular site-of- action and lacks a high affinity receptor. Additionally, plant responses to ToxB require prolonged exposure to the toxin.

Session 5: Effector trafficking: secretion/delivery by microbes
Chair: Francis Martin

5.1 Investigating the delivery of effector proteins by the rice blast fungus *Magnaporthe oryzae*

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Plant pathogenic fungi deliver proteins directly into plant cells to facilitate tissue invasion and to suppress plant defence, but how the fungus delivers these effector proteins during plant infection is currently unknown. We are studying the processes of polarised exocytosis and endocytosis during plant infection by the rice blast disease-causing fungus *Magnaporthe oryzae*. Interestingly, our preliminary data suggests that the site of effector secretion may be distinct from the normal polarised tips of fungal hyphae. We are also studying the role of the *MgAPT2* gene in effector delivery. *MgAPT2* encodes a P-type ATPase in *M. oryzae*, which is required for both foliar and root infection by the fungus, and for the rapid induction of host defence responses in an incompatible reaction. We have explored the relationship between *MgAPT2* and the yeast *DRS2* gene in detail and investigated the role of *MgAPT2* in protein delivery during pathogenesis. In parallel, we are using comparative genomics to define the repertoire of effector-encoding genes in *M. oryzae* in greater detail. We are also investigating endocytosis during plant infection and the potential role of eisosome organelles which localise to specialised domains on the plasma membrane, where they are thought to function in membrane remodelling, and the spatial regulation of endocytosis. We have functionally characterized putative eisosome components in *M. oryzae* andused target gene-deletion to genetically dissect the role of eisosome-associated proteins in this important plant pathogenic fungus, and fluorescent protein fusions to demonstrate the differential localisation of these proteins during infection-related development. In spores of the rice blast fungus, a Pil1-GFP fusion protein localises to punctate patches at the cell periphery, in a pattern consistent with that of eisosomes. Interestingly, the spatial distribution of Pil1-GFP is radically different in vegetative hyphae and invasive hyphae, which are used by the fungus to proliferate within living plant tissue. This suggests that endocytic mechanisms may be distinct in these two developmental stages.

5.2 Bacterial effector delivery

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The type III secretion injectisome is a nanosyringe that injects bacterial effector proteins straight into the cytosol of eukaryotic cells. It is related to the flagellum, with which it shares structural and functional similarities. It consists of a basal body made of several rings spanning the bacterial membranes, connected by a central tube. On top of the basal body, comes a short stiff needle terminated with a tip structure. Three of these rings can assemble and be functional even when their subunit is fused to a fluorescent protein. The combination of these hybrid proteins with an array of mutations in all the injectisome components allowed to decipher the order of assembly of the basal body by fluorescence microscopy. The basal body is assembled sequentially by the Sec pathway. As soon as the export apparatus itself is assembled, it takes over the assembly process and exports the needle subunits (early substrates). Needle elongation is controlled by YscP, acting as a molecular ruler or a timer, released at the end of the process. YscP seems to be partially folded and its total length approximates the length of the needle plus the basal body, supporting the ruler model. According to experiments carried out with partial diploids, only one ruler determines the length of a needle. When assembly of the needle is complete, the C-terminal domain of YscP interacts with the export apparatus and changes the substrate specificity, which becomes ready to export the needle tip protein, then pore formers and finally effectors. One protein from the export apparatus specifically recognizes the various classes of export substrates.

5.3 How oomycete and fungal effectors enter host cells

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Pathogens of both plants and animals produce effectors and/or toxins that act within the cytoplasm of host cells to suppress host defenses and cause disease. Effector proteins of oomycete plant pathogens utilize an N-terminal motif, RXLR-dEER, to enter host cells, and a similar motif, Pexel ($RxLx^{E}/D/_{Q}$), is used by *Plasmodium* effectors to enter erythrocytes. Host cell entry by oomycete effectors does not require the presence of any pathogen encoded machinery. We have found that oomycete RXLR-dEER motifs, as well as the *Plasmodium* Pexel motifs, are responsible for binding of the effectors to phosphatidyl-inositol-3-phosphate (PI-3-P) and/or phosphatidyl-inositol-4-phosphate (PI-4-P). Stimulation of host cell entry by PI-4-P, and inhibition by inositol 1,4 diphosphate or by the phosphoinositide-binding domain of the protein VAM7, support the hypothesis that phosphoinositide binding mediates cell entry. Effectors of fungal plant pathogens were found to contain variants of the RXLR-dEER motif which can enable cell entry. While some fungal effectors bound phosphoinositides, many others bound different phospholipids, consistent with independent convergent evolution of this entry mechanism. Effectors from all three kingdoms could also enter human cells, suggesting that phospholipid-mediated effector entry may be very widespread in plant, animal and human pathogenesis. Inhibition of both plant and human cell entry by the endocytosis inhibitor tyrphostin A23 and the localization of effector-GFP fusions to endosomes in human cells support the hypothesis that entry occurs by receptor-mediated endocytosis.

Session 6: Plant targets of microbes/bioagressor effectors
Chair: Nick Talbot

6.1 Localisation and function of *Phytophthora infestans* RXLR effectors and their host targets

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Eukaryotic plant pathogens, like their better-characterised prokaryotic counterparts, secrete an array of effector proteins that manipulate host innate immunity to establish infection. Deciphering the biochemical activities of effectors to understand how pathogens successfully colonize and reproduce on their host plants has become a driving focus of research in the fields of fungal and oomycete pathology. AVR3a, the first effector characterized from the oomycete pathogen of potato and tomato, *Phytophthora infestans*, was found to contain N-terminal RxLR and dEER motifs required for its translocation across the host plasma membrane. Genomic resources have allowed large-scale computational screening for this conserved motif to reveal >450 *P. infestans* RXLR-EER effectors. We are cloning *RXLR* effector genes to investigate their roles in virulence, their localisation in plant cells and to determine whether they are recognised by host resistance proteins. Yeast-2-hybrid and bimolecular fluorescence complementation are being used to investigate effector-target protein interactions, and to localize these during infection. I will present our progress in the investigation of pathogenicity functions of selected RXLR effectors, including the consequences of stable silencing of these effectors in the pathogen, and data showing the host proteins with which they interact. A range of approaches, including virus-induced gene silencing, are being used to determine the roles of host targets in defence.

6.2 *Xanthomonas perforans* effector proteins as predictive indicators of durable and sustainable resistance to bacterial spot disease of Tomato

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Data will be presented on the identification and characterization of bacterial (TTSS) effector proteins that occur in several naturally occurring field isolates of *X. perforans.* It is generally accepted that the genetic complement of effector proteins delivered to the host via the bacterial TTSS allows the pathogen to suppress or manipulate the host innate immune system, resulting in pathogen multiplication and disease in susceptible hosts. A comprehensive understanding of effector function in *X. perforans* will ultimately reveal the molecular mechanisms controlling virulence in the bacterium and resistance interactions that occur between this bacterium and its host tomato. The ability to rapidly and inexpensively determine the genome sequence of natural field isolates of *X. perforans* from infected tomatoes will provide novel insights into the evolution of pathogen virulence and the allelic diversity of effector genes in natural populations. The knowledge gained from these studies will also provide a comprehensive understanding of the role effector proteins play in bacterial pathogenicity in *X. perforans* and provide the foundation to identify novel sources of resistant germplasm in wild species of *Solanum* to control this disease in a durable and environmentally sustainable manner. Our progress towards these goals will be presented.

6.3 Small RNA pathways and their interference by pathogens in eukaryotes

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RNA silencing is a pan-eukaryotic gene regulation process whereby small interfering (si)RNAs and micro (mi)RNAs produced by Dicer-like enzymes repress gene expression through partial or complete base-pairing to target DNA or RNA. Besides their roles in developmental patterning and maintenance of genome integrity, small RNAs are also integral components of eukaryotic responses to adverse environmental conditions, including biotic stress. Until recently, antiviral RNA silencing was considered a paradigm of the interactions linking RNA silencing to pathogens: Virus-derived sRNAs silence viral gene expression and, accordingly, viruses produce suppressor proteins that target the silencing mechanism. However, increasing evidence shows that endogenous, rather than pathogen-derived, sRNAs also have broad functions in regulating plant responses to various microbes. In turn, microbes have evolved ways to inhibit, avoid, or usurp cellular silencing pathways, thereby prompting the deployment of countercounterdefensive measures by plants, a compelling illustration of the never ending molecular arms race between hosts and parasites. Several original illustrations of these various aspects will be provided, using examples from ongoing studies in our laboratory.

6.4 Nematode effector proteins: Targets and functions in plant parasitism

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Parasitism genes developmentally-expressed in three enlarged secretory gland cells of sedentary endoparasitic nematodes encode multiple effector proteins that are secreted through the nematode's stylet (oral spear) to facilitate the worm's migration within plant roots and mediate the transformation of selected root cells into elaborate permanent feeding cells. Some nematode parasitism genes encode effectors with similarity to known proteins that are involved in cell-wall degradation, peptide signaling, altering cellular metabolism, protein degradation, and nuclear localization, but the majority (>70%) of the predicted effector proteins are unique to these microscopic obligate biotrophs. Examples include a novel nematode effector peptide that interacts with plant SCARECROW-like transcription factors and dramatically increases root growth, a cellulose-binding protein that interacts with a plant pectin methylesterase to condition host cell walls for parasitism, and a functional mimic of plant CLAVATA3/ESR-like peptides that appears to interact in signaling pathways that effect plant cell differentiation. The identification and functional analysis of the effector proteins is revealing the complex nature of the secretions that make a nematode a plant parasite.

Session 7: Microbial effectors in symbiotic interactions
Chair: Sophien Kamoun

7.1 Secretome of the basidiomycete *Laccaria bicolor* and the ascomycete *Tuber melanosporum* reveal evolutionary insights into ectomycorrhizal symbiosis

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Plants gained their ancestral toehold on dry land with considerable help from their mycorrhizal fungal symbionts. The genetic mechanism of this kind of symbiosis contributes to the delicate ecological balance in healthy forests. The genomic sequence for two representative of symbiotic fungi, the Basidiomycota *Laccaria bicolor* and the Ascomycota *Tuber melanosporum*, have been released. We bioinformatically identify >200 candidate genes coding for effector-like secreted small secreted proteins (SSPs) in each of the genomes of *L. bicolor* and *T. melanosporum*, several of which are only expressed in symbiotic tissues and/or fruiting body. Both symbionts thus secrete effector-like molecules that may facilitate the colonisation of their hosts, but expression of several of the secreted small secreted proteins is also upregulated during fruiting body development suggesting a complex interplay between SSPs. In *L. bicolor*, the most highly expressed secreted protein MiSSP7 accumulates in the Hartig net hyphae colonizing the host apoplast. RNAi-inactivation of MiSSP7 showed that this gene has a decisive role in the establishment of the symbiosis. Whether some of these SSPs are similar to those found in other fungi during hyphal fusion and homing, and aggregation of hyphae leading to the formation of sexual organs, remains to be investigated. The unravelling of these secretomes provides tantalizing hints about differences between symbiotic fungi and their saprotrophic and pathogenic relatives.

7.2 Fungal signals and plant fungal perception in the arbuscular mycorrhizal symbiosis

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Arbuscular mycorrhizal (AM) fungi form a mutualistic symbiosis with the root of most vascular plants. This mycorrhiza association is evolutionarily dated as one of the oldest fungal-plant symbiosis on earth reflecting the success of this interaction. Amazingly, it is perhaps one of the most obscure associations due to the genetic intractability of the fungal partner. Thus, while enormous advances on the knowledge about plant perception and accommodation of AM fungi has been achieved in the last years, not so much is known about the details governing the life cycle of the fungus. In our group we are interested in understanding how AM fungi talk to plants and persuade them of their good intentions. With a combination of several molecular approaches we are aiming to identify the chemical signals that trigger plant fungal recognition during the AM symbiosis. We have identified a family of putative effector proteins that are secreted and able to enter the plant and travel to the nucleus. Expression of these proteins *in planta* appears to increase the susceptibility to mycorrhiza formation, indicating that they might play a role in silencing the immune response of the plant. We are currently investigating how the plant perceives these and other fungal signals leading to the activation of the symbiotic program.

7.3 The role of effector proteins in the legume-rhizobia symbiosis

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Rhizobia form symbiotic associations with leguminous plants. The symbiosis begins with an exchange of molecular signals between the two organisms. Flavonoids exuded by plant roots induce the synthesis of Nod factors (NFs) by rhizobia. NFs induce the formation of new plant organs (nodules), into which rhizobia are released. Within nodules rhizobia reduce atmospheric nitrogen to ammonia, which is taken up by the host plant in return for photosynthates. Although Nod factors are essential for nodule formation, there are other determinants, such as secreted proteins, that influence the extent of the symbiosis. Certain rhizobia possess protein secretion systems generally associated with pathogenic bacteria. Pathogens use these systems to inject “effector” proteins into the cytoplasm of their eukaryotic hosts. *Rhizobium* species NGR234 has a functional type III secretion system (T3SS) that is induced by flavonoids and translocates effectors into legume cells. Depending on the legume host, the T3SS can improve or reduce the symbiotic ability of NGR234. NGR234 translocates several effectors; some are members of families of effector proteins found in pathogenic bacteria of both animals and plants, and generally have negative effects on nodulation by NGR234. Molecular characterisation has shown they have similar properties to their pathogenic homologues. Whereas other NGR234 effectors are specific to T3SS-possessing rhizobia and generally have positive effects on symbiosis.
Our working hypothesis is that NGR234 may have acquired a T3SS and adapted it to aid the process of nodulation through the development of specific “rhizobial effectors”. Although relics of the original system may betray NGR234 as a potential pathogen to some plant species, initiating a plant defence response that blocks the symbiotic interaction.

Session 8: Emerging Effectors – nematodes, insects, metabolites
Chair: Sophien Kamoun

8.1 Nematode effectors a genome wide survey

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The Root Knot Nematode (RKN) *Meloidogyne incognita* is a widespread and polyphagous obligate asexual endoparasite of plants that causes serious and growing problems to agriculture. This lifestyle implies dramatical changes of plant cells into complex feeding sites, which are accomplished by effector molecules secreted by the nematode, so-called parasitism proteins. An integrated approach of molecular techniques has been used to functionally characterize nematode parasitism proteins. Very recently, the complete genome sequence of this nematode has been achieved. The assembled sequence of *M. incognita* spans 86 Mbp, and mostly consists of homologous but divergent segment pairs that might represent former alleles in this species. A combination of different processes could explain this peculiar genome structure in *M. incognita*, including polyploidy, polysomy, aneuploidy and hybridization, all features that are frequently associated with asexual reproduction. Another interesting feature of the genome is the spectacular presence of an extensive set of plant cell wall-degrading enzymes in this nematode, which has no equivalent in any animal studied to date. This suite of enzymes likely modify and subvert the host environment to support nematode growth. Initial analyses show that these enzymes are not found in other metazoan animals and their closest homologs are bacterial, suggesting that these genes were acquired by multiple horizontal gene transfer events.

8.2 Using pathogen effectors to investigate host resistance mechanisms

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Plant pathogens use small molecules and also proteins to render their hosts susceptible. Many bacteria and other pathogens use a specialized secretion system to deliver proteins into host cells that interfere with host defence. We have taken advantage of the bacterial type III secretion system (T3SS) to investigate effectors from filamentous pathogens such as oomycetes. We are using T3SS delivery of oomycete effectors from *Pseudomonas* sp to investigate the effector complement of the downy mildew pathogen *Hyaloperonospora parasitica (Hpa)*. I will report recent data on *Hpa* effector functions and on the use of the Solexa/Illumina sequencing instrument to advance our understanding of *Hpa* pathogenicity. We are using Illumina paired read sequencing and Velvet software (Zerbino and Birney, Genome Research, 2008) to assemble sequences of multiple races of another oomycete pathogen, *Albugo candida*, which is particularly effective at shutting down host defence. The analysis of its effectors is likely to provide very interesting new insights into host defence mechanisms. In addition, we are using T3SS delivery of oomycete effectors to investigate the molecular basis of pathogen/host specificity and non-host resistance. An update on recent progress will be presented.

8.3 RNAi knockdown of insect salivary proteins

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Speaking only somewhat in jest, an aphid salivary gland can be thought of as a plant pathogen with legs. That is, it is proteins and enzymes of aphid saliva that, on the insect side, determine whether an interaction with a plant is successful or not, participating at every stage of interaction and continuing throughout feeding. The importance of proteins and enzymes of aphid saliva has been fully recognized for many years, but it is only in the past several years that we have begun to enumerate and know, in any detail, the components of aphid saliva, much less begun to evaluate individual proteins/enzymes as effectors. I will summarize work from several laboratories, including mine, on current, ongoing efforts to catalog the components of aphid saliva (focusing on pea aphid, the model system) using both transcriptomic and proteomic approaches. Then I will turn to our work on Protein C002, the first salivary component for which there is direct evidence in support of its role as an effector (Mutti *et al*. PNAS 105: 9965 (2008)), and two other recombinantly expressed proteins of saliva that we are currently working with.

8.4 Secondary metabolites as effectors: Fungal secondary metabolism is an essential component of the complex interplay between rice and *Magnaporthe grisea*

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Functional analyses of fungal genomes are expanding our view of the metabolic pathways involved in the production of secondary metabolites. These genomes contains a significant number of genes encoding key biosynthetic enzymes such as polyketides synthases (PKS), non-ribosomal peptide synthases (NRPS) and their hybrids (PKS-NRPS), as well as terpene synthases (TS). *Magnaporthe grisea* has the highest number of such key enzymes (22 PKS, 8 NRPS, 10 PKS-NRPS, and 5 TS) among fungal plant pathogens, suggesting that this fungal species produce a large number of secondary metabolites. In particular, it has 10 hybrid PKS-NRPS that likely produce polyketides containing a single an amino-acid. Three of them (*ACE1, SYN2* and *SYN8*) have the same expression pattern that is specific of early stages of infection (appressorium-mediated penetration), suggesting that the corresponding metabolites are delivered to the first infected cells. *M. grisea* mutants deleted for *ACE1* or *SYN2* by targeted gene replacement are as pathogenic as wild type Guy11 isolate on susceptible rice cultivars. Such a negative result could result from a functional redundancy between these pathways. However, *ACE1* null mutants become specifically pathogenic on resistant rice cultivars carrying the *Pi33* resistance gene compared to wild type Guy11 isolate that is unable to infect such rice cultivars. Introduction of a Guy11 wild type *ACE1* allele in *Pi33* virulent *M. grisea* isolates restore their avirulence on Pi33 resistant rice cultivars, showing that *ACE1* behaves as a classical avirulence gene (AVR). *ACE1* differs from other fungal *AVR* genes (proteins secreted into host tissues during infection) as it likely controls the production of a secondary metabolite specifically recognized by resistant rice cultivars. Arguments toward this hypothesis involve the fact that the protein Ace1 is only detected in the cytoplasm of appressoria and is not translocated into infectious hyphae inside epidermal cells. Furthermore, *Ace1-ks0*, an *ACE1* allele obtained by site-directed mutagenesis of a single amino acid essential for the enzymatic activity of Ace1, is unable to confer avirulence. According to this hypothesis, resistant rice plants carrying *Pi33* are able to recognize its fungal pathogen *M. grisea* through the perception of one fungal secondary metabolite produced during infection. The map based cloning of the *Pi33* rice gene was initiated and this gene maps at a locus rich in classical NBS-LRR resistance genes. Further work is ongoing to identify which gene is *Pi33*. In order to characterize the secondary metabolite produced by *ACE1*, this gene was expressed in a heterologous fungal host such as *Aspergillus oryzae* under the control of an inducible promoter. The removal of the three introns of *ACE1* allowed the expression of the enzyme in *A. oryzae*. Characterization of the novel metabolite produced by *Ace1* is in progress.

Poster Abstracts

Listed alphabetically by first author, presenting author is underlined

1. Towards the characterization of a quantitative resistance to downy mildew in cultivated Sunflower, *Helianthus annuus*

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Quantitative resistance to sunflower Downy Mildew caused by the oomycete *Plasmopara halstedii* was studied on a population of recombinant inbred lines (RIL) not carrying efficient major resistance gene, in fields naturally infested by one race of the pathogen (703 or 710). The major quantitative trait locus (QTL) localized on linkage group 10 explains almost 40% of variation, and is not linked to any of the known race-specific resistance genes called PI genes. This QTL confers resistance to at least 2 different downy mildew races and its support interval is 5 cm long. We constructed and screened a BAC library of the RIL parent (XRQ) having the QTL with the closest genetic markers in order to build a BAC contig in the QTL region, a first step towards the positional cloning strategy. The polymorphic BAC ends are currently being used as new genetic markers on the RIL population. We also screened an F2 population of 3500 plants in order to increase the number of plants presenting a recombination event between the closest QTL markers. The evaluation of the resistant phenotypes of such recombinant plants may help restricting the QTL support interval. In order to characterize the expressed genes during the interaction from both partners, plant and oomycete, we initiated a cDNA sequencing approach of infected sunflower plantlets using the 454® sequencing method.

2. Identification of genes putatively involved in manipulating plant defense responses during rice blast infection

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Phytopathogenic fungi have evolved different strategies to proliferate and cause disease. One of the mechanisms used by these pathogens involves the delivery of a battery of effector proteins into the host cell which act to subdue defense responses by interacting with host proteins. In this investigation, three genomic sequences of *M. oryzae* were analysed, 70-15, Y34 and 131 in order to identify genes putatively undergoing diversifying selection. The dN/dS ratio was estimated for each pairwise comparison using Codeml and the twenty five best candidate effectors were identified with a dN/dS > 1. Five genes which had a dN/dS>1 were selected for functional characterization. In a second approach, comparative secretome analysis was carried out to identify proteins specifically present only in ascomycete phytopathogenic fungi. An isochorismatase motif was found in the secretome of five different species of phytopathogens. Isochorismate is a precursor of salicylic acid which is actively involved in plant defense, so it is worth speculating that secreted isochorismatase could be a virulence factor employed by the fungus to reduce salicylic acid. Here we report on the functional characterization of an isochorismatase encoding gene *ISM1* in *M. oryzae*.

3. Arabidopsis downy mildew avirulence locus *ATR5* contains single or multi copy, highly polymorphic non-RXLR effectors among pathogen isolates

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The intricate genetic dance between plant pathogens and their hosts involves the pathogen attack, host defence and the pathogen-counter attack with the use of secreted molecules. Pathogen effector molecules perform inter- and intracellular tasks as adaptation factors and manipulators of the defence network. The *Arabidopsis-Hyaloperonospora* pathosystem has been playing a significant role in uncovering major complementary effector-receptor genes. Common conserved regions including RXLR and EER motifs in the secreted effectors have been identified from several oomycete pathogens, and have been under detailed investigation. Arabidopsis La-*er* accession carries *RPP5*, which recognizes *ATR5* from Noks1/Noco2 and Emoy2 isolates. We have mapped *ATR5* using F_2 mapping populations derived from different crosses between isolates of *H. arabidopsidis*. A genetic interval for *ATR5* has been established and a physical map of *ATR5* in the Emoy2 genome was constructed using the publicly available genomic and BAC-end sequences, as well as the BAC contig data. The *ATR5* gene has been placed on a single BAC clone. Fine mapping has put the gene to a 25kb interval. There is segmental gene duplication in the Emoy2 genome at the *ATR5* locus. Bioinformatic studies supported by expression analysis revealed the presence of five genes, three of which have the characteristics of polymorphic effector molecules in isolate Emoy2. Interestingly, none of these candidates have an RXLR motif. Transient expression studies using bombardment assays have identified the $ATR5^{Emoy2}$ that gives an *RPP5* dependent defence response. The other polymorphic effectors, $ATR5L1^{Emoy2}$ and $ATR5L2^{Emoy2}$, are not recognized by RPP5. We made a cosmid library from isolate Noks1 and identified the clone that covers the *ATR5* locus. Sequence information shows no gene duplication at the locus and only one copy of the putative non-RXLR effector is present. However, this Noks1 copy does not trigger an RPP5-dependant defence response. Analysis of the Cala2 genome using RACE PCR suggested 5 copies of this family of effectors. Recent work on the function, evolution and further analysis will be presented.

4. Fungal cell wall and secreted proteins: insights from the *Tuber melanosporum* genome

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Fungal cell wall is a dynamic structure that plays crucial roles in maintaining cell morphology, protecting mycelia from environmental stresses, and allowing interactions with substrates and the other living organisms. Formation and remodelling of the fungal cell wall involves numerous pathways and the concerted actions of many proteins within the fungal cell. Starting from the genome sequencing project of *Tuber melanosporum*, an ectomycorrhizal fungus, we performed an *in silico* analysis mostly focusing on cell-wall related genes. The results gave us a glimpse on cell wall-related and secreted proteins, allowing the identification of the several members in some gene families (CHSs, chitinases, hydrophobins). Interestingly, arrays expression data suggest that two chitinase genes could be involved in the ectomycorrhizal formation. These proteins could have a role in the cell wall remodelling during the switch from free-living to symbiotic status, but we could also hypothesize a role in the formation of chitin-derived elicitors. A set of genes coding for secreted enzymes involved in a subtle degradation of plant cell wall polysaccharides have been also identified. Future work will be focused to understand whether multiple members of the same gene family have specialized roles

in one or more biological processes (e.g. ectomycorrhizae, fruiting bodies development).

The Tuber genome sequencing project is a collaborative effort involving the Génoscope-CEA (coordinator: P. Wincker) and the Tuber Genome Consortium (coordinator: F. Martin).

5. Unraveling the mode- and site-of-action of the host-selective toxin Ptr ToxB

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Pyrenophora tritici-repentis is a necrotrophic ascomycete and the causal agent of the disease tan spot of wheat. Ptr ToxB (ToxB) is one of the proteinaceous host-selective toxins produced by this pathogen and is responsible for the development of chlorotic symptoms in susceptible cultivars. ToxB is encoded by a multicopy gene, *ToxB* (261 bp), whose expression results in an 87 amino acid (aa) pre-protein. This pre-protein contains a signal peptide of 23 aa and the remaining 64 aa encode the mature form of the toxin (6.5 KDa). There are no characterized motifs within ToxB to give clues to its function site. An allele of *ToxB*, *toxb*, is found in non-pathogenic isolates and encodes an inactive protein. Construction of chimeric proteins containing ToxB and toxb coding regions, and site-directed mutagenesis based on the aa sequence differences have provided information on the structural requirements for ToxB activity. A Proteinase protection assay using heterologously expressed ToxB indicated that ToxB must be present in the apoplastic space for 8 hours to induce maximum symptom development. Barley Stripe Mosaic Virus-mediated transient systemic expression of ToxB and toxb is consistent with the hypothesis that ToxB acts as an apoplastic effector.

6. A functional genomics approach to elucidate the role of aphid salivary gland proteins in plant infestation

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Aphids are amongst the most devastating hemipteran sap-feeding insects of plants. They induce extensive feeding damage and vector the majority of described plant viruses worldwide. *Myzus persicae* (green peach aphid) is considered a generalist, with host plants in over 40 plant families. The *M. persicae* salivary glands probably produce effector proteins that are secreted into the plant host during aphid feeding and modulate plant cell processes. Our aim is to identify and characterize these proteins and to elucidate the molecular mechanisms underlying their functions. Genomics resources recently became available offering unprecedented opportunities for investigating aphids and the perturbations they cause in plants. We applied a data mining strategy combined with functional assays to identify and functionally characterize secreted salivary gland proteins from *M. persicae*. We identified 115 proteins from a salivary gland EST database (3233 ESTs) that are predicted to be secreted. Currently, we are screening this set of proteins for effects on aphid survival, fitness and host range specificity using *in planta* over-expression followed by aphid challenge as well as RNAi in aphids. In addition, we are using transient over-expression assays in *Nicotiana benthamiana* to investigate whether these proteins affect plant cell processes, especially those involved in defense.

7. Structure/function studies of *Phytophthora* effectors using a medium-throughput approach

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Phytophthora species are plant pathogens responsible for massive economic and agricultural losses as they infect crops such as potatoes and tomatoes (*Phytophthora infestans*) or soybeans (*Phytophthora sojae*). They manipulate host cell structure and function through delivery of an array of effector proteins. The RxLR and the CRN (Crinkler) proteins form the two main families of *Phytophthora* effectors. Although many of these effectors can be associated with a virulence and/or avirulence function and phenotype in the host plant, deciphering their biological function remains difficult as they do not share any sequence similarity with proteins of known function. Determining the three-dimensional structure of these proteins would provide significant insights into their function and help direct further biological studies.
Here we describe a medium-throughput approach that was used for the design, cloning, expression and purification of a set of *Phytophthora* effectors. This set contains RxLR and CRN proteins whose expression is known to be induced on interaction with the host plant and have a demonstratable phenotype. The purified effectors will ultimately be used for structure determination by X-ray crystallography as well as biochemical and biophysical assays.

8. Identification of oomycte effector targets using in planta co-immunoprecipitation

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Oomycete pathogens deliver a variety of effector proteins into plant host cells to suppress defense responses and enable successful colonization. In this study, we aimed to find target proteins of the 52 validated oomycete effectors including several with avirulence activity (RXLR family). We used an in vivo co-immunoprecipitation (co-IP) assay to identify the targets of our effectors. We made expression constructs by replacing the secretion signals with the Flag tag and cloning into pJL-TRBO, a binary plasmid derived from a modified Tobacco mosaic virus. We delivered effector constructs into the leaves of *Nicotiana benthamiana* and transiently overexpressed them by agroinfiltration. We then harvested the leaves 2-3 days after infiltration and extracted total proteins. Effector proteins and their interactors from the plant were co-IPed with anti-FLAG resins. Eluted proteins were then run on SDS-PAGE, gel slices were excised, digested with trypsin, and identified by LC-MS/MS. Accepted proteins were required to have Mascot scores of more than 50 and two or more unique peptides identified. We are using reverse co-IP and split YFP assay and to confirm interactions. We will report and discuss identified effector target proteins and any alterations in plant immunity resulting from overexpression or virus-induced gene silencing of these targets.

9. Role of the TtsI protein of *Bradyrhizobium elkanii* in T3SS (type three secretion system)-mediated protein secretion and soybean nodulation

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Bradyrhizobium elkanii is an important soil bacterium which fixes nitrogen and induces nodule formation in soybean (*Glycine max*). The plant-rhizobia interaction is fundamental for the establishment of the symbiosis. Several bacteria release factors that act as elicitors for this interaction, such as Nops (nodulation outer proteins) secreted by type three protein secretion systems (T3SS). TtsI is the transcriptional activator of the system, recognizing consensus sequences (*tts*-box) in the promoter regions of the T3SS genes. To study the *B. elkanii* TtsI protein an omega cassette was used to disrupt the *ttsI* gene, generating a *B. elkanii ttsI* mutant. The mutant and wild type bacteria were used in soybean nodulation and protein secretion assays. Two soybean cultivars were used: in the cultivar Peking a nodulation delay was observed for the mutant strain, while no difference between the wild type and mutant strains was observed for the cultivar McCall. Using Genistein as an inductor for the T3SS, no protein was secreted by the mutant strain. In contrast, the wild type showed a positive western-blot signal against NopA and NopL antibodies. To date, this is the first record of activation of the T3SS in *B. elkanii* by a specific flavonoid.

10. The *Ralstonia solanacearum* GMI1000 effectome

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Ralstonia solanacearum, the causal agent of bacterial wilt disease, targets more than two hundred species including economical important crops. The type III secretion system plays a major role in its pathogenicity. Seventy four type III effectors have been identified in strain GMI1000. To date, 48 have been experimentally validated either through *in vitro* secretion assays or demonstration of translocation into the plant cell. We suspect substantial functional overlap among this repertoire since only two single disruption mutants were found to be slightly altered in pathogenicity on Arabidopsis or tomato plants. In order to get insights into the functions of this large set of effectors, we have defined a systematic functional approach using *in planta* transient expression assays to test the responses induced at the macroscopic level and the localization in the plant cell. The first results show that eight out of the 30 effectors tested induce the development of a necrosis in *Nicotianae*. Most of the effectors-RFP fusions are observed in both the nucleus and the cytoplasm except for three that are exclusively nuclear-localized. For the most promising candidates, plant interacting partner proteins will be searched.

11. Mechanism of cell-death suppression by the *Phytophthora infestans* RXLR effector protein AVR3a

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Phytophthora infestans effector protein AVR3a belongs to the RXLR class of cytoplasmic effectors. AVR3a induces R3a-mediated hypersensitivity and suppresses the cell death induced by *P. infestans* INF1 elicitin, a protein with features of pathogen-associated molecular patterns (PAMPs). AVR3a mutants that activate R3a but do not suppress cell death were identified suggesting that distinct amino acids condition the effector activities. One example is AVR3a^{Y147del} mutant, which lacks cell death

suppression activity but retains R3a activation. These data point to a model in which AVR3a interacts with one or more host proteins. To identify candidate virulence targets of AVR3a, our collaborators in the Birch and Michelmore labs found that AVR3a interacts with the E3 ligase CMPG1 in yeast-two-hybrid assays. Interestingly, AVR3a stabilizes CMPG1 *in planta* whereas AVR3a^{Y147del} does not. CMPG1 is required for INF1-induced cell death suggesting that it could mediate the virulence activity of AVR3a. Our goals are to characterize the AVR3a-CMPG1 interaction and to determine its contribution to INF1 cell death suppression. Currently, we are mapping the interaction sites between AVR3a and CMPG1 and testing the extent to which AVR3a suppresses different aspects of the PAMP-triggered immunity elicited by INF1.

12. Identification of *Phytophthora cactorum* genes expressed during infection of strawberry

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The oomycete *Phytophthora cactorum* causes crown rot disease in strawberry, resulting in big economic losses. To unravel the molecular mechanisms that are involved in the pathogenicity of *P. cactorum* on strawberry, two strategies were followed, SSH cDNA library and effector specific differential display. Two cDNA libraries were made, enriched for *P. cactorum* genes upregulated during infection of strawberry or genes expressed in *in vitro* germinating cysts (a developmental stage essential for infection). Recent characterization of oomycete AVR/effector genes revealed that they encode proteins with conserved RxLR-dEER motifs required for translocating these effectors into host cells. The presence of such a conserved “tag” has provided a tool for discovering the otherwise structurally diverse effector genes. To select RxLR effector genes from *P. cactorum* differential display was performed on eight cDNA populations, including four developmental stages (mycelium, sporangium, zoospore and germinating cyst) as well as four time points during infection (0, 3, 5, 7 days post-inoculation), using the RxLR and EER motif degenerate primers. Using these strategies several genes potentially relevant for pathogenicity, including several putative effector genes were discovered, and their differential expression confirmed using real-time quantitative PCR.

13. Secretion of fungal effectors: a comparative view between a symbiotic and a pathogenic fungus

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In a forest ecosystem, trees are in continuous interaction with different microorganisms including fungi. Among these fungi, some of them are symbiotic as *Laccaria bicolor* while others are pathogenic as *Melampsora larici-populina*. These two organisms are biotrophic fungi. Development of a functional biotrophic interface between fungus and poplar *Populus trichocarpa* needs active secretion of fungal effectors. The recent sequencing of both *L. bicolor* and *M. larici-populina* genomes combined with “transcriptomic” analyses allowed us to establish a genomic view of secretion pathway(s) within both fungi. In addition, we started functional analysis of P4-ATPases family in both fungi as members of this family are involved in endo/exocytosis (1) and secretion of one avirulence gene in *Magnaporthe grisea* (2).Preliminary results will be presented

(1). Graham TR. 2004. *Trends in Cell Biology* 14: 670–677.
(2). Gilbert *et al.*, 2006. *Nature* 440: 535–539.

14. Insights into the *Pseudomonas syringae* pv. *tomato* DC3000 type III effector repertoire gained through combinatorial deletions of effector genes and identification of interacting tomato proteins

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Pto DC3000 uses the type III secretion system to inject ca. 28 Avr/Hop effector proteins into plants, which enables the bacterium to grow from low inoculum levels to produce bacterial speck symptoms in *Arabidopsis thaliana* and the *Solanaceae* species, tomato (*Solanum lycopersicum*) and (when lacking *hopQ1-1*) *Nicotiana benthamiana.* The effectors are collectively essential but individually dispensable for pathogenesis. To understand the basis for this redundancy and the potential function of the effector repertoire as a system, we have been constructing and analyzing DC3000 mutants with combinatorial effector gene deletions and using yeast two hybrid screens to comprehensively identify tomato proteins that interact with DC3000 effectors. Combinatorial deletions involving the 18 effector genes occurring in clusters and two of the remaining effector genes revealed a redundancy-based structure in the effector repertoire, such that some deletions diminished growth in *N. benthamiana* only in combination with other deletions. Much of the ability of DC3000 to grow in *N. benthamiana* was found to be due to five effectors in two redundant-effector groups (REGs), which appear to separately target two high-level processes in plant defense: perception of external pathogen signals (AvrPto and AvrPtoB) and deployment of antimicrobial factors (AvrE, HopM1, HopR1). Similarly, analysis of tomato proteins in the effector interactome has revealed multiple cases of common interactors for two or more effectors. Deletions of complete gene sets for various type III substrates (translocators, lytic transglycosylases, and effectors) in combination with coronatine biosynthesis genes, is revealing the redundancy groups and minimal requirements for each stage in type III effector-mediated pathogenesis.

15. Role of the bacterial Type III Secretion System (T3SS) in the interactions between bacteria and ectomycorrhizal fungi

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In forest ecosystems, exists a mixed fungal-bacterial continuum at the interface between soil and tree roots, called the ectomycorrhizal complex which controls plant health and nutrition. In order to develop new strategies for a sustainable management of forest ecosystems within the forest microbial communities, a better understanding of the interaction mechanisms between bacteria and ectomycorrhizal fungi in the ectomycorrhizal complex is necessary. We hypothesize that the Type Three Secretion System (T3SS), a key secretion-translocation apparatus used by gram-negative bacteria to colonize animal and plant hosts, mediates protein translocation between bacterial and fungal cells and modulates the functioning of the ectomycorrhizal complex. Up to now, T3SS studies have largely focused on bacterial pathogens of animal and plants. However there is increasing evidence to suggest that plant and animal pathogenesis is not the primary function of the T3SS and that the ecological functions of T3SS are more diverse than expected[1]. We recently demonstrated that the Mycorrhiza Helper Bacterial

strain *Pseudomonas fluorescens* BBc6R8, isolated from a sporocarp of the ectomycorrhizal fungus *Laccaria bicolor* S238N, harbours a T3SS gene cluster. Experiences are running to study the functionality of the *Pseudomonas fluorescens* BBc6R8 T3SS and to analyse its role of in the interactions with *L. bicolor*.

16. LecRK79, a putative virulence target of the RXLR effector IPI-O is involved in cell wall-plasma membrane adhesions and PAMP-triggered immunity

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The potato late blight pathogen *Phytophthora infestans* secretes many RXLR effectors, one of which is IPI-O. In IPI-O the RXLR motif overlaps with the tripeptide RGD (RSLRGD), a typical cell adhesion motif present in extracellular metazoan proteins that play a role in cell-cell interactions. A phage display aimed at selecting proteins that potentially interact with the RGD motif in IPI-O resulted in the identification of Arabidopsis lectin receptor kinase LecRK79 (Gouget *et al*. 2006, Plant Physiology 140, 81-90). We postulate that LecRK79 is a virulence target of IPI-O. For functional characterization we generated Arabidopsis lines overexpressing LecRK79 (OE lines) or lacking LecRK79 (knock-out lines *lecrk79).* Infection assays revealed changes in phenotype upon infection with *Phytophthora brassicae* in both the OE and *lecrk79* lines and demonstrate that LecRK79 has a role in *Phytophthora* disease resistance. To unravel the mechanisms underlying this resistance we analysed callose deposition upon PAMP treatment and investigated the strength of cell wall-plasma membrane adhesions by inducing plasmolysis. The results indicate that LecRK79 plays an important role in the continuum between cell wall and plasma membrane and suggest that this lectin receptor kinase is involved in PAMP-triggered immunity.

17. Seven in Absentia (SINA) E3 ligases affect SYMRK protein stability and function in rhizobial entry during *Lotus japonicus* root nodule symbiosis

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SINA E3 ligase proteins are part of the proteasomal degradation pathway, acting as dimers to specifically ubiquitinate their substrates. In *M. truncatula*, they function in regulation of lateral root formation and rhizobial infection (1). Symbiosis Receptor Kinase (SYMRK) activity and phosphorylation is required during root symbiosis to allow internalisation of the microsymbionts. Yeast two-hybrid analysis revealed an interaction of the SYMRK kinase domain with a small family of *L. japonicus* SINA family members, a specific interaction that was confirmed *in planta*. The SINA genes are expressed throughout the plant, and regulated through posttranslational modification and turnover via self-ubiquitination. Protein stability of SYMRK was reduced upon transient co-expression of SINA4 and SYMRK in *N. benthamiana* leaves, indicating that proteasomal degradation of SYMRK is triggered via SINA4. Ectopic expression of a dominant negative mutant form (SINADN) in *L. japonicus* transgenic plants inhibits SINA function, and these plants showed impaired nodulation on the level of the infection process. Our data provide evidence for a role of SINA in rhizobial entry via regulation of SYMRK, which probably involves ubiquitin-mediated internalization and subsequent proteasomal degradation.

(1) Den Herder, *et al*., 2008. *Plant Physiology* 148: 369–382.

18. Identity, host range, vector and spread of a phytoplasma causing tomato stolbur disease in Turkey

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This study aimed to identify the pathogen and determine host range, vector and spread of the causal agent of stolbur like disease on tomato in Turkey. Between 2004 and 2008, plant and pest samples collected from diseased tomato fields in Bursa and Canakkale were examined by nested PCR using phytoplasma-universal 16S rDNA based primer sets (P1/P7). A unique1.4 kb PCR amplified rDNA band from all parts of diseased tomato plants, except seed, were demonstrated that the phytoplasm (PLO) was the causal agent of stolbur disease in tomato. The data showed that stolbur disease was causing epidemic in tomato production areas in the provinces of Bursa (Karacabey and Yenisehir) and Canakkale (Biga) in Turkey. *Cuscuta campestris*, *Orobance ramose*, *Datura stramonium, Polygonum persicaria*, *Setaria* spp., *Chenopodium album* and *Amaranthus albus* were determined as alternative hosts of tomato PLO. Only *Tyhlocyba quercus* among the 22 insect species was found to be potential vector of tomato PLO. All the tomato cultivars/genotypes were found to be susceptible to tomato PLO. RFLP analysis of the PCR-amplified 16S rDNA indicated that all samples contained a closely related phytoplasmas. Phylogenetic analysis of 16S rDNA sequences (1.4 kb) clustered tomato phyoplasmas into a distinct phylogenetic lineages.

19. *Hyaloperonospora arabidopsidis* (*Hpa*) effector's are able to suppress PTI in *A. thaliana*

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We investigate how this obligate oomycete (*Hpa*) is able to manipulate its host, *A. thaliana* to establish a successful infection. We are characterizing *Hpa* effector proteins in collaboration with the ERAPG *Hpa* Effectoromics consortium. These effectors are small secreted proteins containing signal peptide and RxLR motifs. Through bioinformatic analysis of the *Hpa* Emoy2 race genome we identified ≈ 140 potential effectors of which 102 have been cloned. We tested if these effectors (69 now) are able to increase susceptibility in any of 12 *A. thaliana* ecotypes to the virulent bacteria *Pseudomonas syringae* (*Pst*) using a Heterologous (EDV) System (Sohn *et al*., Plant Cell 2007). We also analyzed if the effectors having a positive effect on plant susceptibility are able to suppress PTI, using the *Pst* ΔCEL strain and callose deposition suppression as a marker. We found that most of *Hpa* effectors are able to increase susceptibility to *Pst*. 87% of the *Hpa* RXLRs enhance *Pst* virulence on at least one accession, and 57% on at least 3 accessions. By the contrary, 66% of *Hpa* RXLRs decrease *Pst* virulence on at least one accession, 19.6% on 3 or more accessions. Also, 30% of the candidate RXLRs that enhance virulence do not decrease virulence on any Ecotype. These effectors can likely be targeting host Susceptibility Factors. There is also a positive correlation between enhanced susceptibility to the bacteria and callose deposition suppression by a given effector, i.e.: 78% of the Hpa effectors that could enhance bacterial growth in planta were also able to suppress at least 50% of the callose deposition triggered by Pst ΔCEL, indicating that PTI suppression might be a major mechanism for this pathogen to successfully invade its host.
Only 18% of the tested effectors that do restrict bacterial growth are able to provoke HR-like lesions on the specific *A.t.* accessions that recognize them. When introduced in a non host species (Turnip) most of the effectors have no effect. Only 15% of the Hpa candidates seem to be recognized in a mild way and only 3% strongly, and 17% can enhance Turnip susceptibility to Pst.

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20. Direct effects of AvrPtoB on transcription in Arabidopsis: similarities and differences compared with the pathogen *Pseudomonas syringae* pv*. tomato*

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We report results from microarray experiments designed to identify the effects of AvrPtoB induced in the Arabidopsis accession Niedersenz (Nd-1) and changes caused by the pathogen *P.s.* pv *tomato* DC3000. Data from Affymetrix chips were analysed using Rosetta resolver. Genes differentially affected by wild type DC3000 and the type three secretion system-compromised *hrpA* mutant were identified 2, 4 and 12h after inoculation. Dexamethasone (Dex)-induced expression of the effector AvrPtoB in transgenic Nd-1 caused numerous changes within 2h of induction, some of which were attributed to the Dex treatment. AvrPtoB-specific modifications to the transcriptome were selected. We have found that the effector alone causes a subset of the changes triggered by DC3000. Some of the genes modified are known to have key roles in basal defence, whereas others may impact on nutrient release. We suggest that AvrPtoB has a direct effect on gene expression in addition to its known ability to inactivate certain plant kinases.

21. Small-secreted proteins lost in large A+T-rich isochores as effectors in *Leptosphaeria maculans*: the case study of *LmCys2*

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Leptosphaeria maculans, a phytopathogenic ascomycete causing stem canker of oilseed rape (*Brassica napus*), develops “gene-for-gene” interactions with its host plant where fungal avirulence (*AvrLm*) genes are the counterpart of plant resistance (*Rlm*) genes. Genomic regions around the avirulence genes *AvrLm1*, *AvrLm6* and *AvrLm4-7* were sequenced (1,4 Mb) and found to display a particular organization, with small (20 – 70kb) G+C-equilibrated, ORF-rich isochores intermingled with large (170-450 kb) A+T-rich isochores mainly composed of truncated and degenerated retrotransposons and containing a low density of genes. The three avirulence genes were found in A+T-rich isochores as solo genes: they encode small secreted proteins (SSP) with no homology in the databases and share the same expression kinetics. SSP from A+T-rich isochores are then considered as potential effectors. *LmCys2*, a gene located as a solo gene in the middle of an A+T-rich isochore and encoding a SSP of 247 aa with 8 cysteines, share the same characteristics as the *L. maculans* avirulence genes: a low G+C content, no homology in the databases, specifically found in the *L. maculans* ‘brassicae’ sub-species, the same expression kinetics with over-expression during the primary leaf infection. Functional analyses, including expression silencing or complementation, strongly suggest a role for *LmCys2* in pathogenicity of *L. maculans* and confirm that A+T-rich isochores could shelter genes involved in pathogenicity or avirulence.

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22. Functional analysis of putative secreted nuclear localised-effectors during the *Magnaporthe*–rice interaction

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The filamentous ascomycete *Magnaporthe oryzae* is a hemibiotrophic fungus that causes a devastating disease known as rice blast. The availability of genome sequence from *M. oryzae* has enabled potential effectors to be identified. We mined the complete genome sequence of *M. oryzae* strain 70-15 for the presence of genes encoding secreted proteins based on the presence of N-terminal signal peptides. These secreted proteins are candidate effectors potentially involved in modulating response defences and cellular processes of host plant cells. Three putative nuclear localised-effectors with a DNA binding domain were selected for functional characterization: 1) MGG_03438 with a fungal specific transcription factor domain (FTF); 2) MGG_04326 with a fungal Zinc(2)-Cys(6) binuclear domain (ZnCys); and 3) MGG_13165 with a centromere binding protein B domain (CenpB). To test the role of these putative effectors during rice blast disease, Δ*ftf*, Δ*zncys* and Δ*cenpB* knockout mutants were generated in the Guy11 background. These mutants display wild-type phenotypes regarding root and leaf pathogenicity, vegetative growth and sporulation. To study effector secretion *in planta*, these putative blast effectors were fused to green fluorescent protein (GFP) and are currently being characterised.

23. Cloning and functional analysis of *Phytophthora infestans* RXLR effector, *Avr2*

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An important research goal in the fight against potato blight is to identify pathogen effector proteins likely to be secreted during infection and translocated into host cells to manipulate host metabolism and defence responses. The first effector cloned from *Phytophthora infestans,* Avr3a was found to contain an N-terminal RxLR and dEER motif now demonstrated to be required for transport across the host plasma membrane. Developing sufficient genomic resources has allowed large-scale prediction of oomycete effectors and computational screening for this conserved N-terminal RxLR and dEER motifs has revealed around 500 rapidly diverging effectors.
Our group is currently using the gateway system to clone candidate *P. infestans* RxLR dEER effectors that are expressed during plant infection or with predicted PFAM domains. We aim to develop resistance in potatoes by studying effector function in manipulating host metabolism and defence responses. Plant host interactors of these effectors are being found using Yeast 2-Hyrid assays and gene silencing techniques are being used in *P. infestans* and host plants to investigate their role during pathogen infection.
We would like to present our progress in the investigation of the *P. infestans* effector, Avr2, its recognition by R2-like resistance proteins and its localization in host cells.

24. The E3-ligase effector AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants

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A key feature of active defence mechanisms is the ability to discriminate between self and non-self upon microbial infection. In higher eukaryotes, microbial presence is detected directly by conserved pathogen-associated molecular patterns called PAMPs through a set of pattern recognition receptors (PRRs) which are typically localized in the plasma membrane. Pathogens secrete effector proteins to inhibit signalling events and enhance virulence; for example, the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000) secretes some thirty effectors into the cell via a specialised type-III secretion apparatus. The function for most of these effectors remains unknown but it is generally believed that most of them may interfere with PAMP-triggered immunity (PTI) to overcome resistance. Here we show that the *Arabidopsis* LysM receptor kinase CERK1 plays an essential role in restricting bacterial growth on plants and congruently, it is targeted by *Pto* DC3000 effectors. AvrPtoB blocks all defence responses through this receptor, ubiquitinates the CERK1 kinase domain *in vitro*, and targets CERK1 for degradation via the vacuole *in vivo*. Our results reveal a new pathway for plant immunity against bacteria, and a role for AvrPtoB E3 ligase activity in suppressing PTI.

25. Genomic and proteomic analysis to identify regulatory mechanism of Type III Secretion System in *Pseudomonas syringae* pv. *tabaci* strain IBB1

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Pseudomonas syringae is a model plant pathogenic bacterium which uses Type III Secretion System (TTSS) to inject effector proteins into host cells. In our previous studies, using LC-MS-MS/MS profiling, we have identified a strain of *P. syringae* pv. *tabaci* (referred to as IBB1) that is capable to secrete 17 effectors in culture, while other *P. syringae* strains tested secrete only a few effectors at the same conditions. To find factors contributing to this phenomenon whole genome sequence of the strain of interest was determined by 454 Life Sciences (Roche) technology. The assembling process yielded genomic sequence with near 100% coverage based on *P. syringae* pv. *phaseolicola* 1448A sequence as a reference. Comparison of the genomic sequences of the both strains revealed very strong similarities. We found, however, differences in the sequences coding for proteins belonging to TTSS regulatory machinery. Preliminary investigations indicate that those differences can underlie the capability of strain IBB1 to secrete large number of the effectors in the inducing medium.

26. Dynamics of the changes of electrophysical properties of *Azospirillum brasilense* SP7 cells at their binding with wheat germ agglutinin

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The electrooptical (EO) properties of *Azospirillum brasilense* Sp7 cell suspensions, have been studied at a specific interaction with wheat germ agglutinin (WGA), using the dependences between the changes of optical densities of cell suspensions at the electric orientation of cells and the orienting field frequencies of 740, 1000, 1450, 2000, and 2800 kHz. It was shown that the EO properties of cell suspensions changed at the interaction of cells with WGA and that the EO signal value changed irrespective of the

cultivation conditions. The dynamics of the changes of the EO properties of microbial suspensions was different for microbial cells grown under different conditions. It may be evidence of the differences in the cell surface properties of microbial cells, and of the dependence, between bacterial response to lectin and growth conditions. The possibility of using the EO analysis of bacterial suspensions for the study of the high-specific binding of polypeptide molecular signals with the bacterial target cells and for assessment of the dynamics of this process has been demonstrated.
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27. AvrAC, a *Xanthomonas campestris* pv*. campestris*-specific type III effector, triggers ETI in Arabidopsis vasculature

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Xanthomonas campestris pv. *campestris* (*Xcc*) is the causal agent of black rot on Brassicae and causes disease on cabbage and Arabidopsis for instance. The *avrAC* gene encodes an *Xcc*-specific type III effector which is responsible for avirulence on Arabidopsis ecotype Col-0 exclusively when *Xcc* is inoculated in the leaf vasculature. PCR and dot-blot hybridization performed on a large collection of plant pathogenic bacteria revealed that *avrAC* is specific to *Xcc*. The analysis of more than 50 *Xcc* strains reveals that *avrAC* displays a very low allelic diversity and belongs to the *Xcc* variable effectome. Moreover, the presence of *avrAC* is tightly correlated with an increase in *Xcc* aggressiveness on susceptible Arabidopsis and nonhost pepper plants. We show that the “Leucine-Rich Repeat” (LRR) and “Filamentation induced by cAMP" (Fic) domains are both required for avirulence on resistant Arabidopsis and aggressiveness on pepper. Interestingly, the Fic domains of the VopS and IbpA effectors from animal pathogens were recently shown to mediate protein adenylylation, a yet unknown protein posttranslational modification. Strategies developed to dissect *avrAC* functions *in planta* and to study plant vascular immunity will be presented.

28. Functional mapping of pore-forming harpin HrpZ of *Pseudomonas syringae*: oligomerization and interactions with membrane lipids and plant defence

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Bacterial pathogen *Pseudomonas syringae* is using the type III secretion system (T3SS) to inject plant defence-suppressing effector proteins into the plant cells. HrpZ is a T3SS helper protein, abundantly secreted to the intercellular space, from where it supposedly inserts into the plant cell membrane. In vitro, HrpZ is able to form pores in lipid vesicles. Now we found that HrpZ has a specific affinity to a membrane lipid phosphatidic acid, which suggests that the insertion of HrpZ into the membrane may include a step of phosphatidic acid recognition. We also found that HrpZ forms dimers and higher oligomers with a regular assembly. To map the sites mediating the different molecular interactions of HrpZ, we constructed a full series of deletion mutants of HrpZ of *P. syringae* pv. *phaseolicola* (Pph). The main oligomerization interface mapped to a region near the C-terminus. The same C-terminal region is also required for pore-formation, suggesting that the pores are formed by oligomers. In some non-host plants HrpZ functions as an elicitor of defence responses. To fine-map the elicitor activity, synthetic

peptides representing fragments of HrpZ(Pph) were tested on tobacco for HR induction. A near C-terminal 24 amino acid fragment proved a potent HR-elicitor. Thus, a functionally indispensable part of HrpZ is recognized by tobacco plant to raise defence against the invading bacteria.

29. Genome-wide analysis of secreted proteins encoding genes in *Melampsora larici-populina*

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The foliar rust caused by *Melampsora larici-populina* is the main disease affecting poplar plantation in Northern Europe with severe economic losses. In the wake of the *Populus* genome sequencing, the ~100 Mb genome of *M. larici-populina* have been sequenced (7X depth) by the Joint Genome Institute (JGI, Department of Energy, USA). The analysis of this genome is a great opportunity to identify loci coding for small-secreted protein (SSP) produced by the rust fungus to penetrate and exploit its host. The genome sequence of *M. larici-populina* has revealed a large arsenal of approximately two thousand secreted proteins, half corresponding to SSP ($\leq$ 300 aa). Similarities with effectors previously described in Pucciniales were also uncovered, such as homologs of Haustorially Expressed Secreted Proteins and avirulence factors from *Melampsora lini* or of the Rust Transferred Protein RTP1 from *Uromyces fabae*. Expression profiles of these homologs were monitored by qPCR in *M. larici-populina* during infection of poplar leaves, showing a preferential expression after haustorium formation. Transcriptome Analysis based on 454-pyrosequencing and NimbleGen systems oligonucleotide arrays allowed to identify transcripts encoding for SSP specifically and highly expressed during parasitic growth. Such approaches led to the identification of candidate rust effectors for which functional characterisation through immunocytolocalisation and *in planta* expression is ongoing.

30. Transcriptomics of nematode effectors

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Root-knot nematodes (RKN) are obligate root parasites that establish an intimate relationship with the host plants and induce the re-differentiation of root cells into specialized feeding cells called giant cells. In order to identify the nematode effectors involved in the fine manipulations of plant defences and plant cell fate, we focused on nematode genes induced during parasitism. We focused on *Meloidogyne incognita,* from which the genome has been recently sequenced. Nematode genes induced during the interaction were isolated by the construction of subtractive libraries from the free-living J2 and mixed parasitic J3 and J4 stages (Dubreuil *et al*., 2007). A second strategy was the *in silico* clustering analysis of EST obtained from free living and parasitic stages available in public and local databases. The local database contained 67 615 new EST sequences for *M. incognita*. Full-length coding sequences were obtained by EST clustering and identification of the corresponding gene models in the genome. Candidate effectors were selected based on the presence of a predicted signal peptide for secretion, the presence of functional domains in the deduced proteins and the search for orthologs in the genome of free living and parasitic species.

31. *Pseudomonas syringae* HopI1 effector targets plant Hsp70 for virulence

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Pseudomonas syringae uses type III secretion to inject many proteins into plant cells for a successful infection. The HopI1 effector targets chloroplasts and suppresses salicylic acid accumulation and related defenses. HopI1 has a C-terminal J domain and a P/Q-rich repeat region. J domains typically interact with and stimulate the activity of Hsp70 proteins. Here we show HopI1 acts as a co-chaperone of Hsp70 by increasing its ATP hydrolysis activity. HopI1 directly interacts with a host Hsp70 via its J domain, as shown by *in vitro* binding, co-IP from chloroplast extracts and LC-MS/MS analysis. While the virulence function of HopI1 depends on interaction with Hsp70, this is not sufficient since HopI1ΔP/Q still binds Hsp70 and targets chloroplasts, but is not active in virulence. HopI1's virulence effect is severely compromised in plants with reduced Hsp70 levels. We hypothesize that HopI1 may prevent Hsp70 from folding/stabilizing a defense factor and/or may subvert Hsp70's function to mimic a stress response. The link between virulence and stress is suggested by the observations that HopI1 confers thermotolerance to plants and the virulence defect of a Δ*hopI1* strain is overcome by infecting plants at elevated temperature.

***32. Streptomyces scabies* NahG counteracts salicylic acid mediated defense responses in plants**

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The Gram-positive Plant pathogen *Streptomyces scabies* causes scab disease on potato and other root and tuber crops. Thaxtomin, a nitrated dipeptide phytotoxin, inhibits cellulose biosynthesis and is indispensible for *Streptomyces* pathogenicity. The *S. scabies* 87-22 genome encodes a putative salicylate hydroxylase (NahG) which is predicted to be secreted and is transcribed in a plant mimic medium. A Δ*nahG* mutant of strain 87-22 is reduced in virulence on Arabidopsis relative to the wild-type strain. When expressed under the 35S promoter in Arabidopsis, *Streptomyces* NahG-YFP fusion protein localized in the plant cytoplasm. Arabidopsis over-expressing the *Streptomyces* NahG were more susceptible to both *S. scabies* and *Pseudomonas syringae*. Interestingly, we found that salicylic acid (SA) suppresses thaxtomin production *in vitro* and down regulates transcription of the thaxtomin biosynthetic genes. Based on these results, we propose that NahG functions in catabolism of elevated SA in *S. scabies*-infected plant tissue, thus mitigating SA-induced suppression of thaxtomin biosynthesis.

33. Structure/function characterization of the *Ralstonia solanacearum* type III effector GALA7, controlling host specificity on *Medicago truncatula*

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GALA7 is one of the 71 effectors that are secreted by the type III secretion system *of Ralstonia solanacearum*. Like its six other family members, GALA7 is an F-box-LRR containing protein that is expressed under the control of the *Hrp* regulon. It is injected into the plant cell where it possibly mimics a component of the plant ubiquitine-ligase machinery for the benefit of the pathogen. A subset of GALA proteins is required for virulence of *R. solanacearum* on tomato and Arabidopsis, while GALA7 was found to be a host-specificity factor required and, on its own, sufficient for disease on *M. truncatula.* Moreover, *R. solanacearum gala7* mutant strains could not be complemented with a

GALA7 construct lacking the F-box domain (Angot *et al*., 2006) suggesting that the potential ubiquitine-ligase function is required for virulence on *M. truncatula.*
Both soil-grown and *in vitro*-grown *M. truncatula* plants inoculated with a *gala7* mutant strain are free of symptoms. Colonization of the plants by the *gala7* mutant strain, as tested on *in vitro* inoculated plants, is substantially lower than colonization by the wild type strain GMI1000. The *in vitro* inoculation system is now being used as standard method to perform the structure/function analysis of GALA7.
To functionally characterize GALA7 we used the sequence diversity in *GALA7* that exists between *R. solanacearum* strains from different origin, representative for most phylotypes and subgroups. All newly obtained sequences will be used for enhanced evolutionary studies on GALA proteins. Constructs carrying the different *GALA7* ORFs with and without epitope tags were used to complement *gala7* mutant strains, which were tested for pathogenicity and multiplication in *M. truncatula*. Antibodies raised against GALA7 or the epitope tag were used to check for proper type III dependent secretion. After testing the natural occurring diversity in *GALA7* we plan to specifically test site-directed mutants for complementation. Finally, to further our understanding of the functionality of GALA7 we are screening a *M. truncatula* yeast-two-hybrid library to search for GALA7 plant protein partners.

Angot A, Peeters N, Lechner E, Vailleau F, Baud C, Gentzbittel L, Sartorel E, Genschik P, Boucher C, Genin S. 2006. *Ralstonia solanacearum* requires F-box-like domain-containing type III effectors to promote disease on several host plants. *Proceeding of the National Academy of Sciences, USA* 103: 14620–14625.

34. Effect of Fungal Elicitors on the Glycyrrhizin Production in *Agrobacterium* Mediated Transformed shake flask cultures of *Abrus precatorius* L.

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Abrus precatorius L., (Fabaceae) known as Indian liquorice which is common bedding deciduous vine. Objective of the study is to describe the combination of Ti and Ri plasmid- mediated transformation of *A. precatorius* and fungal elicitation for the enhanced production of glycyrrhizin in the established crown gall and hairy root culture. *Agrobacterial rhizogenes* and *Agrobacterial tumefaciens* were putative hairy roots. The genetic transformation was confirmed by opine assay and PCR analysis. Analysis for glycyrrhizin content was performed by HPLC method. The fungal elicitors prepared from *Aspergillus niger* (MTCC2198) and *Rhizopus stolonifer* (MTCC2594), were tested at different concentrations to enhance glycyrrhizin production in suspension culture of transformed cells. Maximum enhancements of 3.02-fold and 2.83-fold in glycyrrhizin content, respectively, were obtained with *Aspergillus niger* (10%v/v) and *R. stolonifer* (7.5%v/v) 3rd and 6th day after elicitor treatment. The present study reports the first successful genetic transformation and production of glycyrrhizin by transformed cell cultures of *A. precatorius*.

35. A type-II secreted effector of *Pseudomonas syringae* inhibits a distinct plant apoplastic Cys protease

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Arabidopsis cell suspension cultures respond to *Pseudomonas syringae* pv. *tomato* (*Pst*) infection by secretion and activation of a papain-like cysteine protease called RD21. Virulent strains of *Pst* are able to suppress RD21 activity and cause disease. We tested a series of putative cysteine protease inhibitors present in the genome of Pseudomonas. These proteins were over-produced in *E. coli* and the inhibitory effect on plant proteases was investigated using activity-based profiling with DCG-04, a biotinylated derivative of the protease inhibitor E-64. We found that one of the *Pst* proteins is indeed a potent inhibitor of RD21. We named this inhibitor RD21 inhibiting protein 1 (RIP1). Sequence analysis revealed that RIP1 possesses a type-II secretion signal and a lipo-box that anchors the protein in the outer membrane. Phylogenetic studies using RIP1 sequences from different Pseudomonas isolates revealed that RIP1 is in all Pseudomonas species under conservative selection. Modelling experiments show that the few variant amino acids that are localised on the surface of RIP1 are probably not affecting the proposed inhibition mechanism. RIP1 knock-out strains were generated, and found to cause significantly reduced disease symptoms and bacterial growth on Col-0 plants. This phenotype is complemented by constitutive over-expression of RIP1.Taken together we believe that RIP1 plays a crucial role in neutralizing secreted papain-like cysteine proteases at the surface of the bacterial cell wall. This mode of action is unique and will be discussed in detail.

36. Establishment of FLP-dependent recombination in *Ustilago maydis* and its use for the functional analysis of effector gene families

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In the *Ustilago maydis* genome several novel secreted effector proteins are encoded by gene families suggesting a redundant function. Due to the limited number of resistance markers these effector genes families have not yet been functionally analyzed. To solve this problem we have established an inducible FLP-mediated recombination system in *U. maydis*. For multiple gene disruption experiments we have constructed a hygromycin marker gene flanked by two direct copies of *FRT* sequences. This cassette is then flanked by homology arms on either side for individual genes. To eliminate chromosome rearrangements caused by intramolecular recombination between identical *FRT* sites left behind after FLP-mediated marker excision, a set of mutated *FRT* sequences were designed. The FLP-mediated marker recycling was then successfully applied to delete a family of nine genes coding for secreted proteins with unknown function. In plant infection assays we could demonstrate a reduction in virulence in a mutant strain carrying a deletion of six genes and virulence was further reduced when the remaining members of the family were deleted. For one gene we have already established partial complementation of the deletion phenotype, and this is now used to assess the functionality of mCherry fusions to be used for localization studies.

37. A putative novel effector family in arbuscular mycorrhizal fungi

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Biotrophic fungi, interacting with plants, establish long-term relationships with their hosts to fulfil their life cycles. In contrast to necrotrophs, biotrophic fungi need to sort out the defense mechanisms of the plant to develop within the host. A way to achieve this is the delivery of fungal effector molecules to short cut the plant defense program. Arbuscular mycorrhizal (AM) fungi are obligate biotrophs of roots establishing associations that last the life span of the plant. It is now evident that this is only possible through a complex exchange of molecular information. To study the existence of potential effector proteins in AM fungi, we generated several cDNA secretion libraries of *Glomus intraradices* at different stages of the symbiosis. Using the yeast signal sequence trap method, we identified a novel family of secreted fungal proteins containing tandem hydrophilic repeats and a nuclear localization signal. *In planta* expression of the putative effectors showed that indeed they are secreted and targeted to the nucleus. Yeast two hybrid analysis revealed that one of these proteins interacts with components of the alternative splicing machinery. This suggests that these fungal proteins might play a role in manipulating the host cell program to establish the biotrophic status.

38. Proteins secreted by the mutualistic fungus *Piriformospora indica* during *Arabidopsis* root colonization

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The basidiomycete *Piriformospora indica* forms mutualistic symbioses with several mono- and dicotyledonous plants, including *Arabidopsis*, thereby improving plant performance as well as enhancing biotic and abiotic stress resistance and tolerance. Although cyto-histochemical and genetic studies on plant roots revealed a fungal colonisation pattern that apparently depends on root cell death, transmission electron microscopic investigations detected an early biotrophic colonisation phase in the *Arabidopsis-P. indica* interaction preceding the cell-death dependent colonisation phase. This finding implies a more sophisticated communication between the interacting partners in order to establish a compatible interaction. Recent transcriptome analyses even revealed the suppression of the plant innate immune responses by *P. indica. P. indica* is thought to secrete effectors during root colonisation that interfere with the plant innate immune system, modulate host metabolism, and even contribute to cell death regulation. We introduced the yeast signal sequence trap assay and isolated several putative effectors of *P. indica*. We will present the candidates identified by this approach, discuss their probable function during plant colonisation and give first results on their activity.

39. Identification of fungal secreted substances that regulate early mycorrhizal marker genes

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Arbuscular mycorrhizal (AM) fungi form long-term symbiosis with roots of more than 80% of all land plants and are obligate biotrophs. Similar to other biotrophic fungi colonizing plants, AM fungi need to avoid the defense mechanisms of the plant to

develop within the host and feed on living cells. It is an emerging issue that a way to achieve this is the delivery of fungal effectors molecules from earliest fungal life cycle stages that short cut the defense program of the host. It is now evident that this is only possible through a complex exchange of molecular information between both partners that allow them to life in symbiosis.
It has been shown that some *Medicago truncatula* genes are regulated specifically in presence of *Glomus intraradices* even before both organisms contact. Analysis by qRT-PCR of some of those genes will be use as a reporter method to identify fungal secreted substances that acts as plant elicitors. *M. truncatula* roots incubation at different times with fungal exudates and gene expression analysis will be the experimental approach. Differential extraction of those exudates will also help to classify or identify the biochemical nature of those fungal elicitors.

40. Analysis of WRKY transcription factors in barley - powdery mildew interaction

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WRKYs are plant specific transcription factors known to be involved in the regulation of plant defence via activation or repression of promoter activities. In *Arabidopsis*, many WRKY proteins are induced by salicylic acid or pathogens. They bind specifically to the TTGACC/T motif of W-boxes which are over-represented motifs in promoters of genes involved in basal defence or systemic acquired resistance (SAR, Maleck *et al*. 2000) and are also commonly found in WRKY promoters themselves. In barley, we have demonstrated expression of a set of WRKYs upon infection with the biotrophic powdery mildew fungus *Blumeria gramin*is f.sp. *hordei*, *Bgh*. Over-expression of barley HvWRKY1 and HvWRKY2 resulted in increased accessibility of epidermal leaf cells to the pathogen (Eckey *et al*. 2004), indicating WRKY's participation in the early metabolic reprogramming during a compatible interaction. As earlier reported, HvWRKY1 and -2 physically interact with an R-protein activated by a fungal Avr effector *in vivo* (Shen *et al*. 2007). Further analyses of the mode of WRKY action demonstrate suppression of a *Bgh*-induced barley promoter by co-expression of HvWRKY2. This finding is consistent with the proposed function of HvWRKY1/ -2 as plant compatibility factors and repressors of basal defence.

Maleck *et al*., 2000. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. *Nature Genetics*; 26: 403–410.
Eckey *et al*., 2004. Identification of powdery mildew-induced barley genes by cDNA-AFLP: Functional assessment of an early expressed MAP kinase. *Plant Molecular Biology* 55: 1–15.
Shen *et al*., 2007. Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science* 315:1098–1103.

41. Is the cell wall integrity signaling pathway involved in the perception of microbial cellulose-binding effectors?

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Carbohydrate-binding modules (CBM_1) belonging to the family 1, bind cellulose and have been initially found in fungal cellulases where the play a key role in enzyme function by enhancing substrate accessibility. CBM_1 have a huge distribution among oomycetes, where they are present in non-enzymatic cell-surface proteins named

CBELs (Cellulose Binding Elicitor Lectin), found exclusively in oomycetes (Gaulin *et al*., 2008). These proteins play a key role during plant infection by mediating adhesion of the parasite to cellulosic substrate. Strikingly, *Phytophthora* CBM_1 act as MAMP during plant infection by triggering innate immunity in several plant species (Gaulin *et al*., 2006). Experiments with synthetic CBM_1 from various oomycete species revealed their universal ability to induce plant immune responses. CBM_1 unable to interact with cellulose are also impaired in their eliciting activity. Based on these data we have proposed a model linking cellulose alteration to defense induction, where structural modification of the cellulose substrate due to CBM_1 binding could be perceived by a cell-wall integrity sensing machinery (Dumas *et al*., 2009). This model will be presented, in addition to an analysis of CBM_1s evolution and origin in oomycetes which are phylogenetically distant from fungi.

Dumas, B., Bottin, A., Gaulin, E., and Esquerré-Tugayé, M.T. 2008. Cellulose Binding Domains: cellulose-associated defense sensing partners? *Trends in Plant Science* 13: 160–164.
Gaulin, E., Drame, N., Lafitte, C., Torto-Alalibo, T., Martinez, Y., Ameline-Torregrosa, C., Khatib, M., *et al*., 2006. Cellulose binding domains of a *Phytophthora* cell wall protein are novel pathogen-associated molecular patterns. *Plant Cell* 18: 1766–1777.
Gaulin, E., Madoui, M.A., Bottin, A., Jacquet, C., Mathé, C., Couloux, A., Wincker, P., and Dumas, B. 2008. Transcriptome of *Aphanomyces euteiches*: new oomycete putative pathogenicity factors and metabolic pathways. *PLoS ONE* 3 e1723

42. Suppression of plant defense response in Norway spruce by bacterial VOCs

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The aim of this study was to investigate how the volatile organic compounds (VOCs) of streptomycetes influence the immunity of the forest tree Norway spruce. The applied methods included the use of in vitro cultures, light microscopy and gene expression analyses. Previously, we have shown that *Streptomyces* AcH 505 promotes root colonisation by a plant root pathogenic fungus *Heterobasidion abietinum* 331, which was neither due to the substances auxofuran nor WS-5995 B, the water soluble secondary metabolites of *Streptomyces* AcH 505. Using a culture system where the bacterium was separated from plant roots and fungal mycelium, we observed that increased fungal colonisation is due to volatiles produced by AcH 505 although these volatiles did not promote the growth of the pathogenic fungus. Instead, they suppress the expression levels of defence related genes in Norway spruce, leading to promoted root colonisation. Thus, VOCs of AcH 505 influence the complex signalling in Norway spruce rhizosphere and modulate the pattern of secondary infections.

43. Characterizing the role and recognition of ATR13 from *Hyaloperonospora arabidopsis*

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ATR13 is an effector secreted by *Hyaloperonospora arabidopsis* (Hpa) through its haustoria during infection of *Arabidopsis thaliana*. Once secreted by the pathogen, this protein is taken up by an unknown mechanism into the plant cell where it acts to manipulate host defense pathways. Recently it has been shown that ATR13 suppresses callose deposition (1), but how ATR13 does this is not known. ATR13 has no sequence similarity to any known protein, and therefore a molecular function cannot be assigned. X-ray crystallography and NMR spectroscopy are in progress to address this and solve the structure of ATR13, thereby allowing us to compare folds that are potentially

conserved in proteins with known function. Using protein NMR analysis, we have eliminated regions of disorder and are currently working towards obtaining phasing information that will allow us to integrate our x-ray diffraction data. Additionally, work is being done to elucidate the RPP13 resistance pathway known to recognize the presence of certain isolates of ATR13 within a plant cell and mount a defense response. Two separate lines have been mutagenized with EMS and are being screened with Hpa or subjected to selection.

44. Characterization of the elicitor structure of *Potexviruses* inducing the *Rx*-mediated resistance

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Plants are continually challenged by various pathogens. The *Rx*-mediated resistance against *Potato virus X* (PVX) illustrates a defense mechanism known as the gene-for-gene model (Flor 1971), in which the recognition of the PVX coat protein (CP) the elicitor, by the product of the *Rx* gene, a NBS-LRR protein determines the outcome of the interaction (Bendahmane *et al.*, 1995).
Previous studies have shown that the CP of other *Potexviruses* (NMV, CymMV, WClMV and PepMV) can induce the *Rx*-mediated resistance (Baurès *et al.*, 2008; Candresse *et al.*, submitted paper). Since the primary sequence of these CPs reveals less than 40% of identity, the recognition is likely based on a particular structure. Our goal is to characterize the biochemical features of these elicitors. In a first step, a deletion study allowed the identification of a minimal elicitor for several *Potexvirus* CPs. In a second step, the production of the minimal elicitors has been pursued, for PVX and PepMV, in heterologous system and their purification for different physicochemical analysis is in progress.

45. AvrPphB, a jumping effector that travels between bacteria on a mobile genomic island

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The acquisition of genomic islands (GIs) containing effector genes by horizontal gene transfer has played a major role in microbial evolution. There are few practical demonstrations of the acquisition of genes that control virulence and, significantly, all have been achieved outside the animal or plant host. Loss of a GI from the bean pathogen *Pseudomonas syringae* pv. *phaseolicola* (*Pph*) is driven by exposure to the stress imposed by the plant's resistance response. We have developed fluorescent tags to follow loss of the GI from bacteria during infection of bean and also after exposure to plant extracts *in vitro*. Here, we show that the complete episomal island, which carries pathogenicity genes including the effector *avrPphB*, transfers between strains of *Pph* by transformation *in planta* and inserts at a specific *att* site in the genome of the recipient. Our results show that the evolution of bacterial pathogens by moving effector repertoires may be achieved via transformation, the simplest mechanism of genomic exchange. This process is activated by exposure to plant defences, when the pathogen is in greatest need to acquire new genetic traits to alleviate the antimicrobial stress imposed at microsites within challenged leaves by plant innate immunity.

46. Competitive Index analysis of type III effector contribution to virulence in *Ralstonia solanacerum*

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In recent years, many type III secreted effectors have been identified in the sequenced strain *Ralstonia solanacearum* GMI1000, although the contribution to virulence of most of them remains unclear. Functional redundancy between type III effectors has been proposed as a factor hindering the virulence analysis of effector mutants, since the virulence phenotypes of most mutants are usually very slight or undetectable using regular virulence assays. We have previously shown that competitive index assays (CI), are a more sensitive and accurate method to detect growth differences within the plant than standard assays, allowing detection of otherwise undetectable virulence phenotypes in the plant pathogen *Pseudomonas syringae*. In this work, we set the basis for the use of mixed infections as a virulence assay in this strain, and its application to the analysis of GMI1000 effector mutants. Several GMI1000-derivative strains carrying single or multiple mutations in type III effectors were analysed for their virulence in eggplant and tomato plants, and CI analysis has already allowed us to establish growth attenuation for some of them. Interestingly, this approach also allowed to detect a differential contribution of some Type III effectors depending on the nature of the host plant tested.

47. Quantitative contribution to virulence of type III effectors in *Pseudomonas syringae* pv. *phaseolicola*

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In recent years, many type III secreted effectors have been identified in the sequenced strain *Pseudomonas syringae* pv. *phaseolicola* 1448a (Pph 1448a), although the contribution to virulence of most of them remains unclear. Functional redundancy between type III effectors has been proposed as a factor hindering the virulence analysis of effector mutants, since the virulence phenotypes of most mutants are usually very slight or undetectable using regular virulence assays. We have previously shown that competitive index assays (CI), are a more sensitive and accurate method to detect growth differences within the plant than standard assays, allowing detection of otherwise undetectable virulence phenotypes. In this work, we set the basis for the virulence analysis of Pph 1448a effector mutants at different stages of the infection process, using CI assays. We generate many Pph 1448a-derivative strains carrying single or double mutations in type III effectors, and analyse their virulence in bean plants, demonstrating growth attenuation for many of them. We also analyse functional redundancy between effectors, demonstrating growth attenuation for double effector mutants of the same effector family, not detectable in the corresponding single effector mutants.

48. Investigating the delivery of effector proteins by *Magnaporthe oryzae*

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Phytopathogenic fungi have evolved mechanisms to suppress plant defences during infection-related development. They deliver proteins directly into plant cells to facilitate

tissue invasion, but how the fungus delivers proteins during plant infection is currently unknown. *Magnaporthe oryzae* is the causal agent of rice blast disease. Previous analysis has shown that the *MoAPT2* gene, which encodes an aminophospholipid translocase, is involved in secretion of proteins during plant infection and required for the rapid induction of host defence responses in an incompatible reaction. One of the main objectives in this project is to determine whether those effectors are delivered to plant cells in a MoApt2-dependent manner. Fluorescent-tag gene fusion effectors have been created with native signal peptides and promoter sequences and a GFP at the C-terminus. *M. oryzae* effectors are expressed only during invasive growth in rice tissue. Our results showed that these effectors localize to discrete sub-apical aggregates at the periphery of hyphae, suggesting this localization is dependent upon the signal peptide. This analysis demonstrates that these proteins are produced and delivered only *in planta* where they may play, as yet uncharacterized roles in plant defence suppression and could also be important triggers for activating the immune response of the host plant.

49. Investigating the importance of effectors with lysin motifs of the rice blast fungus *Magnaporthe oryzae*

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Magnaporthe oryzae is a filamentous ascomycete fungus and the causal agent of rice blast disease. Over three billion people depend on rice as part of their staple diet, yet millions of tonnes of rice yield are lost each year due to *M. oryzae*. During biotrophic invasive growth, *M. oryzae* delivers a subset of effector proteins in order to evade host defense responses and promote undergo growth, although the mechanisms by which this occurs in plant pathogenic fungi as a whole is still largely unknown. In this study, two putative effector proteins from *M. oryzae* containing lysin motifs, MoLYS1 and MoLYS2, were investigated. Both proteins show significant homology to the known virulence factor Ecp6, which is secreted in to the apoplastic space of tomatoes by the leaf mold fungus, *Cladosporium fulvum* (Bolton *et al*., 2008). Targeted gene replacement of *LYS1* and *LYS2* genes in order to characterize these genes and demonstrate their potential role as virulence factors in *M. oryzae*. The localization of both LYS1 and LYS2 was also investigated using translational fusions to green fluorescent protein (GFP) in order to examine their localization *in planta* and to investigate the cellular localization and secretory mechanism of putative apoplastic effectors.

50. The small effector molecule Syringolin A inhibits the plant proteasome *in vivo* and during infection

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Pseudomonas syringae pv. *syringae* (*Pss*) is a pathogen of snap bean and also highly virulent in *Nicotiana benthamiana*. *Pss* strain B728a secrets the small molecule Syringolin A (SylA) that has been identified as a virulence factor. SylA is a proteasome inhibitor that blocks the activity of yeast and mammalian proteasomes. Direct evidence of *in vivo* activity of sylA in the plant proteasome was still to be elucidated. Activity-based proteome profiling (ABPP) is a powerful technology to study the activity of the plant proteasome that can be applied for *in vivo* studies. ABPP is based on the use of

fluorescent inhibitors that react with catalytic residues of enzymes in an activity-dependent manner. By using ABPP we demonstrate that SylA selectively inhibits the Arabidopsis proteasome *in vivo* and that bacteria producing SylA are able to inhibit the proteasome of *Nicotiana benthamiana* during infection.

51. Putative Effectors of *Moniliophthora perniciosa*, the causative agent of Cacao Witches' Broom Disease

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The hemibiotrophic basidiomycete *Moniliophthora perniciosa* is the causative agent of Witches' Broom Disease of cacao (WBD), a devastating disease that has been damaging cacao plantations in Brazil. The *M. perniciosa* genome project (www.lge.ibi.unicamp.br/vasssoura) was launched aiming to identify genes that could provide clues about pathogenic factors of this fungus. The genome survey of *M. perniciosa* was released, giving new insights about WBD. Among the analyses performed during this initiative, we searched for proteins rich in cysteine containing secretion signal peptides, which are common traits of effectors proteins. Many of those found proteins have no similarity with proteins described in databank. Bioinformatics tools suggested that the one of those new proteins, named MpLRP1 (*M. perniciosa* Lysine Rich Protein), has chitin binding motifs. The gene encoding MpLRP1 was cloned in protein expression vectors. MpLRP1 was then expressed in *E. coli* and purified. Binding assays indicated that this protein has the potential to bind to chitin, a feature similar to *Cladosporium fulvum* effector Avr4.

52. Anatomical changes induced by *Bursaphelenchus xylophilus* in tissues of *Pinus pinaster*

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Bursaphelenchus xylophilus, the pine wood nematode (PWN) has become a serious threat to Portuguese pine forest, since its detection in 1999.
To understand the host reaction during disease development, two-year old *P. pinaster* seedlings were inoculated with PWN and samples, harvested after three, five and seven weeks, were prepared, following standard methods, for histopathological studies.
At "the early stage" of infection, when no external symptoms were yet visible, PWNs were observed within the lumen of the cortex resin ducts. During the "advanced stage" of infection, when external symptoms were plainly visible, severe tissue degradation occurred. Cavities with irregular boundaries developed from degraded resin ducts and surrounding parenchyma cells. As the disease proceeded the cambial zone was degraded and the cavities expanded and fused. At the seventh week after inoculation the number of nematodes has increased dramatically and all pine tissues are severely damaged.

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53. Virulence effectors of *Erwinia carotovora*

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Erwinia carotovora subsp. *carotovora* (*Pectobacterium carotovorum* subsp. *carotovorum*) is a major bacterial pathogen infecting potato and several other crops world-wide. It causes soft rot through the co-ordinate production of plant cell wall degrading enzymes. Although these enzymes are major pathogenicity determinants, studies in recent years have indicated that the disease process in *Erwinia* is complex, and a lot about the molecular mechanisms and regulation of virulence remains to be elucidated. In this project, we analyse the genome sequence of *E. carotovora* subsp. *carotovora* to better understand the molecular basis of virulence in this bacterium, which has a significantly broader host range than the previously sequenced exclusive potato pathogen *E. carotovora* subsp. *atroseptica* (*Pectobacterium atrosepticum*). The genome of *E. carotovora* subsp. *carotovora* encodes unique genes that are not found in the genome of *E. carotovora* subsp. *atroseptica*, such as the plant ferredoxin-like protein gene *ferE*. Comparison of these two genomes will be discussed.

54. Ectomycorrhizal (ECM) community structure: The role of host tree genotype

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It is well established that resistance of forest trees to disease is under genetic control but our knowledge of the importance of host genotypic variation in mycorrhizal formation is currently limited. However, recent studies found marked variation in ectomycorrhizal fungi (ECM) communities among conspecific tree and the ability of tree individuals to form ectomycorrhizas with a single fungal strain has been demonstrated to have a genetic basis. These observations suggest that host genotype is an important factor in determining the associated ECM species composition, which may in turn influence the growth and vitality of the tree host.
We are currently investigating the importance of the genetic component of Norway spruce in determining early formation of ECM symbiosis. The degree of genetic control will be measured as the broad-sense heritability of fungal diversity. Norway spruce trees were sampled for cuttings from Southern Finland last winter. 14-28 rooted cuttings from 55 unrelated trees have been inoculated with the mycelia of five ECM species. In autumn 2009, 10 inoculated and 10 uninoculated cuttings per clone will be sampled for analysis of ECM community. Direct PCR from ECM will be used to enable efficient identification of ECM colonization.

55. Evolutionary perspective from the *Phytophthora infestans AvrBlb2 family*

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Oomycete pathogens secrete a battery of molecules that modulate, alter, and suppress plant defense. One of these groups of protein effectors, the RXLR family, is know to occur in a number of *Phytophthora* genomes. The structure and organization of such group of effectors genes however, may have unique features depending on particular host preference and evolutionary life histories. In *P. infestans*, some RXLR effectors appear to evolve as superfamilies. AVRblb2 is a small (100aa) protein that has been shown to activate Rpi-Blb2-mediated recognition, an R gene recently cloned from *S.*

bulcocastanum. A detailed analysis reveals that AVRblb2 is also part of a large superfamily of proteins that include at least 19 members within the T30-4 genome. Seven of this members are >90% similar to AVRblb2 (paralogous) while the rest share between 63 and 42% of similarity. In this study we track the occurrence of the AVRblb2 family on *P. infestans* and sister species. In addition to expression profiles for these genes, we also test avirulent activity of some AVRblb2 members on Rpi-Blb2 transgenic plants. The contribution of this family to the life history of some *Phytophthora* species is discussed.

56. The effects of bio- and nitrogen-fertilizers on corn, cv. SC704

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A split-plot experiment was conducted based on completely randomized blocks design with four replications in Islamic Azad University, Karaj branch, during 2008. The three nitrogen levels, including zero, 100 and 200 kg N ha^{-1} were in main plots, with five biofertilizers treatments in subplots, including control, Azotobacter + Azospirillum, Azotobacter + Pseudomonas, Azospirillum +Azotobacter, Azotobacter + Azospirillum + Pseudomonas). Results showed that grain and biological yields, 1000 kernels weight, and ear yields per area and per plant were affected by both nitrogen and biofertilizer, and kernel numbers in each ear row and in ear only were affected by applying biological fertilizers. As nitrogen fertilizer was increased, all yield components were improved. Also, applying biofertilizers was superior to control. Interactions between bio- and nitrogen-fertilizers on grain yield, 1000 kernels weight, and kernels in row and ear were significant. Applying Azotobacter + Pseudomonas along with 200 kg N ha^{-1} was predominating over other treatments. However, simultaneous applying of all three bacterium without nitrogen application led to better performance of corn plants compared to applying bacterium with nitrogen, showing non-necessity of nitrogen application in conditions with presence of these bacterium which can reduce chemical fertilizers hazards in a environmental friendly manner.

57. The RXLR effector *PpAvh153*: the first avirulence candidate in the broad host range oomycete *Phytophthora parasitica*

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Phytophthora parasitica is a hemibiotrophic oomycete pathogen that may attack many herbaceous and wooden plants of economical importance worldwide. To date, effective treatment to control diseases caused by *P. parasitica* is limited and resistance breeding has been developed for only a few species including tobacco.
The generation of various *P. parasitica* EST libraries have allowed THE IDENTIFICATION OF several classes of effector proteins among which oomycete avirulence homolgs (Avhs). One of them named *PpAvh153* encodes a potentially secreted RXLR effector protein of 153 amino acids. Association genetics over a >50-isolate sampling from various geographical and host origins, expression profiling, and functional analyses revealed that i) *PpAvh153* is expressed in late but not early steps of tomato infection; ii) strains virulent on tobacco possess multiple copies of *PpAvh153*, all of which carrying frameshift mutations, as potential non-functional alleles; iii) PpAVH153 is able to induce necrosis on tobacco, suggesting that it may represent an avirulence gene towards this plant. *PpAvh153* orthologs were found in the *P. infestans* and *P. sojae* genomes, but not in the other oomycete genomes sequenced so far. The high conservation of this sequence in at least 3 distinct *Phytophthora* species leads to

hypothesize that it may play a key role in the biology, and/or pathogenesis of *Phytophthora*.

58. The HrpN protein, which participates to Type III secretion translocation of the plant pathogen *Erwinia amylovora*, triggers plant basal defense response

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Pathogenicity of the plant pathogen *Erwinia amylovora* relies on a functional type III secretion system (TTSS) which allows translocation of a number of effectors into the plant cell. Injected effectors manipulate the plant cell metabolism to allow successful bacterial colonization. Among those, the DspA/E effector efficiently suppresses plant basal defense response such as callose deposition. Surprisingly, an *E. amylovora tts* mutant is unable to trigger callose deposition indicating that this plant basal defense response is triggered either by another type III effector or by the TTS apparatus. Genetic analysis indicates that the TTS protein HrpN, which is involved in DspA/E translocation, is required to trigger callose deposition. The purified HrpN protein is inducing a faint callose accumulation. HrpN could be injected into the plant cell as shown by analysis of translocation of HrpN1-200::CyaA fusion. Translocation of HrpN1-200-CyaA or HrpW1-200-CyaA fusions is impaired in a *hrp*N background indicating that HrpN involvement to the translocation process is not restricted to DspA/E translocation. Transgenic apple seedlings expressing a HrpN protein directed to the membrane exhibit callose deposition without pathogen challenge. All these data indicate that HrpN participate to the translocation pore and that translocation is a process which activates plant defense response.

59. Investigation of the role of *MYCORRHIZA-INDUCED SMALL SECRETED PROTEIN7* in the formation of poplar ectomycorrhiza

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During root colonization effector like-small proteins secreted by ectomycorrhizal fungi are thought to play an integral role in establishment of symbiosis. Based on the presence of a secretion sequence, the *Laccaria bicolor* encodes 208 predicted Small Secreted Proteins (SSPs) (Martin *et al*., 2008). Using expression profiling of mature *L. bicolor*:Poplar mycorrhiza, we identified 6 SSPs with very high expression levels. One of these genes, dubbed *MYCORRHIZA INDUCED SMALL SECRETED PROTEIN7* (MISSP7), was chosen for further analysis. As has been found with many fungal effectors, *MISSP7* bears no sequence homology to genes from other fungal genomes. Using immunolocalization we show that MISSP7 is localized to the apoplastic space within mature ectomycorrhiza. We have also created *L. bicolor MISSP7* mutants by RNA silencing to assess the impact of transcript inactivation on ectomycorrhiza development. Implications of our findings and the potential role of MISSP7 in mycorrhiza formation will be discussed.

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60. The role of plant immunophilins in host–pathogen interactions

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At the given stage of investigations in the field of plant functional genomics studying of orthologous genes, are being one of the main links in comparative genetics of various organisms. For today a number of genes classes, which products is known, possessing similar primary, secondary and even tertiary structure, carry out similar functions in organisms which are taking place on completely various branches of eukaryotic phylogenetic tree. However also the genes possessing functional homology, and were called orthologous which, nevertheless, essentially cannot possess similar functions in various organisms are identified.
One of the major classes of the genes involved in regulation of a cellular metabolism and the immune answer in human organism are immunophillins. Immunophillins are coding a wide spectrum proteins which are mostly connected to stimulation T-lymphocytes against pathogens. However function of these genes widely presented in *Arabidopsis thaliana* genome, and identified several years ago, till now is not known. By 2008 a number of jobs were published showing the participation of plant immunophillins in the answer on various abiotic and biotic stress factors. FKBP's are the subclass of immunophillins, contain, at least, 8 conserved domains that are presented in various combinations but some combinations of domains in the same protein do not ever meet. So the creation of "a universal gene", by directed mutagenesis in vitro which will allow to combine functional domains in the same protein that are not presented in nature, allowing to increase plant resistance to the different stresses.

61. Production of the effector homoserine lactones by cultivated mushroom pathogens and associated bacteria

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In many bacteria a major level of cell-density response that has been called Quorum Sensing (QS) exists. In gram-negative bacteria a typical QS system is based on the production and response to acetylated homoserine lactones (AHLs). AHL-mediated-QS has considered as potential target for an alternative strategy for bacterial disease control based on its inactivation (Quorum Quenching, QQ). The aim of this study was to evaluate the production of AHLs either by strains of *Pseudomonas agarici*, *P. tolaasii*, *P. gingeri*, *P. reactants* and *Burkholderia gladioli* pv. *agaricicola* or bacteria associated to mushrooms in order to reveal the possible regulation of the disease process by AHLs-QS and then to set out potential mushroom disease control by QQ. Strains of *B.g*. pv. *agaricicola*, *P. agarici and P. gingeri* were demonstrated to produce AHLs. Among 234 bacterial isolates obtained from *A. bisporus*, *P. ostreatus* and *P. eryngii* niches 43 isolates were found to produce an array of AHL analogs based on some biosensor response to diffusible molecules. Furthermore pathogen that produces the elicitor AHLs apparently enhanced disease symptoms in tissue block agaricus test suggesting that AHLs may take part in the pathogenesis regulation of mushroom pathogen.

62. Molecular regulation of two mitogen-activated protein kinases and cyclic-AMP during infection hyphae development in *Heterobasidion annosum*

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The basidiomycete *Heterobasidion annosum (Fr.) Bref*. is a filamentous white rot fungus, considered to be the most economical important pathogen of conifer trees. But very little is known about the molecular and biochemical aspects of the infection process. This study investigates two intracellular pathways that are probably important during the infection structure development in *H. annosum*. The cyclic-AMP/ PKA and the mitogen-activated protein kinases (MAPKs) pathways are known to be important in the signal transduction cascade regulating fungal development and virulence. We investigated the intracellular level of the second messenger cyclic-AMP during the early stage of spore germination characterized by the formation of a primary germ tube followed by hyphae development. Conidiospores were inoculated in medium supplemented with either xylose, glucose or wood extract and the cAMP was extracted and quantified at different time points. The initial results reveal higher cAMP levels during primary germ tube formation in conidiospores exposed to wood extracts compared to simple sugars. In a separate study, the regulation of two MAPK probably involved in osmotic stress tolerance and virulence were investigated. PMK-like and HOG-like MAPKs were cloned and a functional complementation in *S. cerevisiae* mutant strains as well as qRT-PCR were performed. The results will be presented and discussed.

63. Plant proteins targeted by the GALA type III effector family from the pathogenic bacterium *Ralstonia solanacearum*

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Type III secretion system is a major pathogenicity determinant of *Ralstonia solanacearum*. Among the 80 predicted effectors was identified a family of 7 proteins, named GALAs, homologous to eukaryotic F-box proteins. In plants, F-box proteins are components of SCF complexes involved in the ubiquitination process, a key post-translational modification regulating protein stability, subcellular localization or activity. Thus, GALAs could enable *R. solanacearum* to subvert its host ubiquitin-proteasome system.
We have shown that GALAs are collectively required for *R. solanacearum* pathogenicity on *Arabidopsis thaliana* and Tomato, and that GALA7 is a host specificity factor on *Medicago truncatula*.
A yeast two-hybrid screen against an *Arabidopsis* cDNA library showed that GALAs could interact both with ASKs (members of the SCF complexes), suggesting that GALAs are able to form composite SCF complexes *in vivo*, and with a protein potentially involved in vesicular trafficking. Ongoing experiments aim at testing if this protein is indeed a GALA target and investigating the role of this protein during disease development.
GALAs effectors are likely to represent a new example of how pathogens can manipulate the ubiquitin-proteasome system of their hosts. Moreover, identifying the function of GALAs targets will probably bring new insights into the biology of plant resistance / susceptibility to bacterial pathogens.

64. Uptake and transport of *N*-acetyl-homoserine lactones in barley

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Over the last two decades it has become apparent that bacteria are able to communicate with each other via a process named Quorum sensing (QS) and thereby orchestrate bacterial gene expression. QS is also involved in mediating the interaction between different bacterial species and between bacteria and eukaryotic organisms. *N*-acetyl-homoserine lactones (AHLs)are the major signalling molecules in QS of Gram-negative bacteria and it has been shown that these bacterial signalling molecules are able to induce responses in plants including systemic resistance. However transport and translocation into plants remains almost unknown. We used tritium labelled C8-and C10-homoserine lactone (HSL) to analyze the uptake and translocation in barley (*Hordeum vulgare* L. cv. “Barke”) plants within the first 24h after treatment as well as the inhibition of C8-HSL transport in barley roots. Additionally we visualized the AHL transport in the central cylinder in cross sections of maize roots via autoradiography. Despite the fact that the majority of AHLs remains attached to the outer root surface, the plant reacts to the minute concentrations of AHL incorporated with changes in the activity of detoxification enzymes. The consequences for a growing crop are critically discussed.

65. *Arabidopsis* and *Salmonella*, a new model in human pathogens–plant interactions.

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Salmonella enterica serovar *typhimurium* contaminated vegetables and fruits are considerable sources of human infections. Bacteria present in raw nutrients cause salmonellosis, the most prominent food poisoning in the world. This endopathogen enters host cells actively modifying the cytoskeleton and forming entocytotic vacuoles (SCV). Through a set of effectors, these bacteria suppress the host’s immune responses. Although *Salmonella* survives on plants, interactions between plants and bacteria are only partially understood. In this report we study the mechanisms of plant infection. We show that *Salmonella* can enter plant cells and proliferate within. Effectors allowing the entrance and proliferation in mammalian cells are known. Here, we asked the question whether the entry into plant and animal utilize the same set of bacterial proteins. We used two independent strategies to identify the bacterial factors required in infection of plant cells. In the first approach, the known virulence factors and effectors from *Salmonella typhimurium* 14028s were cloned and tested for their action *in planta*. The second strategy uses known Salmonella mutants which present an avirulent phenotype in animals and humans. The data obtained in those two approaches point to similar strategies used by Salmonella during the internalization in animal and plant cells.

66. Identification of a secreted effector of *Sporisorium reilianum* contributing to host specificity

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Sporisorium reilianum causes head smut of maize and sorghum. Mating competent haploid strains fuse to form infectious dikaryotic hyphae that can penetrate young leaves. Although hyphae subsequently invade all plant tissues, sporulation takes place

exclusively in the inflorescence. We isolated haploid strains from spores of diseased maize and sorghum plants. Infection assays showed that maize isolates of *S. reilianum* (M) are infectious on maize but not on sorghum, while sorghum isolates of *S. reilianum* (S) are infectious on sorghum but not on maize. M and S strains are mating competent, and crosses lead to spore formation on both maize and sorghum. Thus, the isolated M and S strains are varieties of the same species.
An infection of sorghum with M strains leads to the formation of red necrotic lesions containing phytoalexins, which suggests that M strains succumb to host defense. To identify genes involved in host selection of *S. reilianum*, we undertook a comparative genomics approach, which led to the identification of a candidate gene (*c1*), encoding a small secreted protein highly conserved among maize smut fungi. Deletion of *c1* in maize smut pathogens led to a reduction of virulence on maize. Accordingly, expression of *c1* in S strains increased virulence on maize. On the other hand, if S strains expressing *c1* were used to infect sorghum, they induced the formation of red lesions. This suggests that *c1* encodes a secreted effector protein involved in determining host specificity of *S. reilianum*.

67. Mycorrhizal features of federally threatened *Vanda testacea* (orchidaceae) with reference to histochemical and confocal approach

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The orchid mycorrhizas grow into orchid tissues and form elaborate coiled structures known as pelotons within cortical cells. The mycorrhizal fungal mycelium is converted into pelotons and these pelotons lyse and the lysed products are absorbed by the host and are designated as digestion cells. Protein content using Mercuric Bromophenol blue stain which stains the lysing pelotons which give intensity of colour depends on different level of lysis. These result indicated that older pelotons contain relatively more proteins that the younger ones, suggesting that the older pelotons are more active during lysis. Relative concentration of RNA which is an index of metabolic activity of the peloton of the host cell was studied using toluidine blue. The variation in intensity of blue color was the index of RNA content, accordingly the higher intensity suggested that older pelotons possessed higher amount of RNA indicating metabolic activity than the younger ones. The host cell DNA continues to increase in size and its affinity for azure B, fast green, methyl green acridine orange, rhodamine B and toluidine blue O increase steadily. The results obtained in this study would be useful in the conservation of the species in the natural habitat.

68. AWR: A *Ralstonia solanacearum* effector family key for virulence and HR

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More than 70 putative Type III Secretion effectors have been encountered in the phytopathogen *Ralstonia solanacearum.* Amongst them, the present work focuses on a multigenic family of 5 members called AWR. These effectors might be crucial for bacterial infection as deletion of one AWR renders *R. solanacearum* GMI1000 less virulent on tomato.
The impact of AWR proteins on the plant physiology was evaluated by *Agrobacterium*-mediated transient expression in *Nicotiana* leaves. Despite of their apparent toxicity under the microscope, we could conclude that AWRs do not localize in the nucleus. This toxicity was corroborated macroscopically after 1–4 days post-induction when leaves

showed a clear necrosis. Although the extent of necrosis differed among AWRs, expression of AWR1024 was giving rise to the strongest one (followed by AWR0099), resembling an HR phenotype. This HR-like phenotype was confirmed by Trypan Blue staining (specific for cell death) and by RT-PCR of specific HR marker genes.
As some AWR effectors are even toxic in yeast, one may think that they interfere with conserved pathways such as central metabolism or cascade signalling. Deciphering the targets of the AWR effectors will unravel the mechanism of action of an effector family that has a dramatic impact on plant physiology.

69. Antimicrobial effects of some volatile oils produced by plants from *Lamiaceae* family

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The main objective of our work consisted in characterization of volatile oils produced by secretory hairs from 2 populations of *Ocimum basilicum* L. and 1 population of *Perovskia atriplicifolia* Benth., and determination of antimicrobial effects of those compounds. The volatile oils extraction was conducted using a Clevenger hydrodistillation system. The component separation was performed by gas chromatography using a 6890 Agilant GC-MS. The volatile compound identification was made using the NIST spectral bank and Kovats indexes. Antimicrobial effects were tested using a difusimetric method and Gram positive and negative bacterial strains. The number of volatile compounds identified in the oil samples is variable, according to the analyzed taxon, the harvesting moment of the samples (the age of the plants), as well as the analyzed organ. The investigated volatile oils have antimicrobial effects on *Staphylococcus aureus* (depending on concentration) and a mild influence on the growth and development of *Escherichia coli*.

70. The role of CRN8, a LQLFLAK effector from *Phytophthora*, during pathogencity

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Phytophthora spp. cause some of the most destructive plant diseases in the world. *P. infestans* is an oomycete, and secretes an arsenal of effector proteins that modulate plant innate immunity to enable infection. Effectors can be classified based on their target site in the host as apoplastic effectors and cytoplasmic effectors. The CRN (Crinkle) family containing the LXLFLAK motif is a relatively unknown class of cytoplasmic effectors. One of the *crn* genes, *crn8*, encodes a secreted protein with a C-terminus containing a predicted serine/threonine RD kinase domain. Secreted kinases have not been reported from microbial plant pathogens, so far. Expression of CRN8 induces cell death. Mutation of the catalytic residues RD into AA abolished cell death induction suggesting that kinase activity is important. CRN8 localizes inside the plant nucleus and nuclear localisation of CRN8 is required for the induction of cell death. CRN8 has a high similarity to a plant kinase-like protein so CRN8 could mimic a specific class of plant enzymes. Obtained results of the CRN8 pathogen effector and the CRN8 plant kinase-like protein will be presented. These new findings will enable us to decipher how pathogens successfully colonize and reproduce on their host plants.

71. Proteome and transcriptome analysis to identify effectors from the nematode *Meloidogyne incognita*

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Sedentary plant-parasitic nematodes are major agricultural pests worldwide. Root-knot nematodes trigger the formation of specialized feeding cells named “giant cells” by substantial reprogramming the developmental process of root cells. Giant cells are generated as a result of the injection of nematode effectors and candidates from *M. incognita* have been identified by proteomic and transcriptomic approaches that possibly regulate plant defence and/or host cell development. I*n vitro* stimulation of secretion by a neuro-stimulant (resorcinol) leaded to the identification of proteins secreted by infective pre-parasitic juvenile nematodes. Among them are enzymes involved in cell wall degradation, detoxication (e.g. glutathione peroxidase, glutathione S-Transferase), signalling (e.g. 14-3-3-like proteins, calreticulin) and others. A Mi-CRT is secreted *in planta* throughout parasitism. Transcriptomic analysis of nematode genes up-regulated during parasitism identified several additional candidate effectors. Among them, *Mi-gst-1* is transcribed in the secretory oesophageal glands of the nematode and harbours a predicted signal peptide motif for protein secretion. *Mi-gst-1* silencing results in reduced nematode egg production. In addition, examples of genes abundantly transcribed in sedentary parasitic stages compared to infective pre-parasitic juveniles encode a cathepsin B-like cysteine protease and a predicted serine carboxypeptidase. Further functional analyses of secreted proteins during parasitism will help to understand how nematodes manoeuvre signalling pathways leading to plant susceptibility and giant cell induction.

72. Sugar transport and virulence in *Ustilago maydis*

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The smut fungus *Ustilago maydis*, a ubiquitous pest of corn, is highly adapted to its host plant to parasitize on its organic carbon sources. We have identified a plasma membrane-localized sucrose transporter (Srt1) from *U. maydis* as virulence factor. Srt1 is the first high affinity sucrose transporter described for fungi and belongs to a previously undescribed class of transporters. The protein is expressed exclusively during infection. The high affinity of Srt1 specific for sucrose allows the direct utilization of sucrose at the plant/fungal interface without extracellular hydrolysis. By that, Srt1 avoids the apoplastic glucose signaling known to elicit plant immune responses. The second sugar transporter required for full virulence of *U. maydis* is the hexose transporter Hxt1. In addition to its function as a high affinity transporter for glucose, fructose and mannose, global expression analysis revealed a role as hexose sensor. Deletion of *hxt1* not only alters expression of metabolic genes, but also that of genes shown to be involved in mating and subsequent pathogenic development. Our data give novel insights into the carbon utilization and signaling during pathogenic development of biotrophic fungi.

73. Transport of the secreted protein NopE1 into nodule cells and its impact on symbiosis

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The type III secretion system (T3SS) is a versatile protein transport machinery, which was first identified in pathogenic bacteria. Within the Rhizobiaceae, T3SSs have been identified in *B. japonicum*, *Rhizobium* sp. strain NGR234, *S. fredii* and others. If effector proteins influence symbiosis positively or negatively depends on the host. Although several type III-secreted proteins have been described, little is known about their function or activity. *B. japonicum* secretes at least nine proteins after induction by genistein. Two of the proteins are NopE1 and NopE2, which are homologous but have no similarity to characterized proteins. A translational *nopE1-lacZ* reporter gene fusion is expressed in mature nodules of *M. atropurpureum* suggesting that the protein is active during symbiosis. The nodulation phenotype of a *nopE1- nopE2* double mutant differed from the phenotype obtained with the wild type or single mutants. Results obtained with *nopE1-cya* fusions suggest that NopE1 is translocated into the cytosol of the host cells. In supernatant of genistein-induced *B. japonicum* cultures NopE1 is present in fragmented form. To test if fragmentation is due to specific or unspecific cleavage, NopE1 was purified from *E. coli*. Further tests revealed that NopE1 exhibits self-cleavage. The cleavage sites are further characterized by Edman degradation and site-directed mutagenesis.

74. Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen *Magnaporthe oryzae*

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To isolate novel avirulence genes from *Magnaporthe oryzae*, the rice blast fungus, we examined DNA polymorphisms of secreted protein genes predicted from the genome sequence of isolate 70-15 and looked for an association with AVR activity. This large-scale study found significantly more presence/absence polymorphisms than nucleotide polymorphisms among 1,032 putative secreted protein genes. Nucleotide diversity of *M. oryzae* among 46 isolates of a worldwide collection was extremely low ($\theta = 8.2 \times 10^{-5}$), suggestive of recent pathogen dispersal. However, no association between DNA polymorphism and *AVR* was identified. Therefore, we used genome resequencing of Ina168, an *M. oryzae* isolate that contains nine *AVR* genes. Remarkably, a total of 1.68 Mb regions, comprising 316 candidate effector genes were present in Ina168 but absent in the assembled sequence of isolate 70-15. Association analyses of these 316 genes revealed three novel *AVR* genes, *AVR-Pia*, *AVR-Pii* and *AVR-Pik/km/kp*, corresponding to five previously known *AVR* genes, whose products are recognized inside rice cells possessing the cognate *R*-genes. *AVR-Pia* and *AVR-Pii* have evolved by gene gain/loss processes, whereas *AVR-Pik/km/kp* has evolved by nucleotide substitutions as well as gene gain/loss. Host target proteins and pathogen-to-host trafficking of the isolated AVRs will be discussed.

75. Functional characterization of LXAR-containing effectors of *Magnaporthe oryzae*

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The majority of the cloned avirulence genes of the hemibiotrophic fungus *Magnaporthe oryzae* encode secret proteins, conserving a common feature of avirulence genes in other filamentous phytopathogens. A relatively conserved motif, designated LXAR, was identified in some of the avirulence genes in *M. oryzae* and shares similarity to the well-characterized RXLR motif in the avirulence genes in *oomycetes.* In *M. oryzae*, we totally identified around 160 LXAR-containing predicted secreted proteins and found that this LXAR motif was significantly distribute at the N-termini in secreted proteins compared with non-secreted proteins. Out of these 160 genes, 45 genes do not have introns and thus were selected for functional characterization with respect to their virulence activity. Interestingly, 27 genes were found to be able to significantly suppress the BAX-mediated cell death in tobacco leaves using agrobacterium mediated transient assay. The effect of cell-death suppression was observed when both full-length and mature proteins were tested. However, mature proteins show stronger effect in cell-death suppression than full-length proteins, indicating that these proteins function inside plant cells. One of the effectors, mag_part2_3018, was further selected to investigate whether the mutation of LXAR motif interferes with the activity of cell death suppression. Interestingly, mag_part2_3018^{AAAA} maintains the activity of cell death suppression as the wild one when the mature protein was tested. On the contrary, mag_part2_3018^{AAAA} was not able to suppress cell death when the full-length protein was tested. These results indicated that LXAR motif is not involved in the activity of cell death suppression. Instead, it might involve in translocation of some effectors into plant cells. Sequence polymorphism and phenotyping of some strains with overexpressed effectors will also be presented.

76. First insight into the genome and the transcriptome of *Piriformospora indica*

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Piriformospora indica is a plant root-colonizing fungus (Basidiomycota) which forms a novel type of mutualistic symbiosis with a wide range of mono- and dicotyledonous crop plants. Phylogenetic analyses showed that *P. indica* is closely related to a terrestrial orchid mycorrhiza complex of *Sebacina vermifera* isolates. All investigated fungi from this group demonstrated host plant growth promotion and increased plant resistance to leaf pathogens such as *B. graminis*. Here we report a first insight into the genome sequence of the archetype *P. indica* and highlight some of the gene sets that may be involved in the symbiosis. With approximately 20 ± 5 Mb genome size and 10,000 estimated genes of which 600 were predicted to be secreted, the genome of *P. indica* is one of the smallest genomes in Basidiomycota. Genome sequence consents now the identification of primary factors that regulate symbiotic development and metabolic activity in plant–*P. indica* interaction. This information will help understanding the role played by endophytic fungi in plant development and physiology, allowing the full ecological significance of this symbiosis to be explored.

77. Identification and functional analysis of *Magnaporthe oryzae* effectors

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The ascomycete *Magnaporthe oryzae* causes 'blast disease' affecting several cereals in particular rice. During the compatible interaction, the first phase of *M. oryzae* infection cycle is biotrophic. *M. oryzae* is suspected to secrete, as bacterial and oomycete pathogens, an arsenal of effector proteins that disrupt the activation and execution of plant defences. In order to identify *M. oryzae* effectors, a search for putatively secreted proteins during rice infection was performed from different sequence datasets such as, genome annotation data, transcriptome data of blast fungus-infected rice leaves, and proteomic data of blast proteins released in culture media. A list of 370 candidate effectors expressed during rice infection and possessing a putative signal peptide for secretion was generated. Among them, 58 where shown to be secreted *in vitro* in proteomic analyses, while the expression of 10 was found to be down-regulated in the *M. oryzae bip1* mutant impaired in a b-zip transcription factor essential for the pathogenesis of the blast fungus. First results of the functional analysis of these candidate effectors will be presented.

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