Epigenetic hotspots as ecological niches for pathogenicity-related genes?

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Unveiling and exploiting *P. capsici* nuclear effector functions

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Plant pathogens are a major agricultural concern hampering food production worldwide. The highly destructive oomycete *Phythophtora capsici* threatens a diverse range of economically important crops such as pepper, tomato, eggplant, snap, lima beans as well as many cucurbits. *Phythophtora capsici* secretes a vast arsenal of proteins (effectors) that are thought to be crucial for its virulence. Work in our lab has identified a suite of *P. capsici* effectors that localise to the host nucleus, nonetheless little is known about the nuclear processes they target.

The aim of my project is then to unveil the activities of *P. capsici* effectors responsible for reprogramming the host nucleus during infection. An interdisciplinary approach that combines Yeast two hybrid (Y2H), proteomics, transcriptomics and functional analyses will be employed to study the functions of effectors and their targets *in vivo*.

By Y2H screening two protein interactors of Crinkler 83_152 (CRN83_152), a nuclear effector from *P. capsici,* were identified: SUMO ligase and endonuclease. Here I will present my results regarding the confirmation and characterization of CRN83_152 interactions.

ARABIDOPSIS INTERACTION WITH BENEFICIAL AND PATHOGENIC MIRCOBES:

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Plants are permanently challenged by a number of adverse environmental factors including a large variety of soil microbes. To adapt to these conditions, plants have developed a plethora of phosphorylation-based sensing and signaling mechanisms that allow optimal coordination of their growth, development and physiological programmes. In terms of the interaction of plants with microbes, the innate immune system revealed to play a key role in regulatig plant growth and defense.
Salmonella typhimurium, one of the major causes of food poisoning and death in humans, is often found in soil samples and can infect and propagate both in animals and plants. Salmonella applies a plethora of strategies to infect plants that are counterbalanced by typical plant defense responses, including the regulation of MAP kinases, NBS-LRR receptors and or lipases. A progress report will be given on SHIPREC (http://www.erasysbio.net/index.php), a European consortium that aims to compare the evolution and functioning of the human and plant innate immune system in response to Salmonella infection.
To further compare the responses of Arabidopsis between detrimental and beneficial microbes, we have started DARWIN21, a large scale project to isolate rhizosphere microbes from plants living in different deserts. DARWIN21 aims to better understand the contribution of rhizosphere microbes to plant survival under extreme conditions of heat, drought and salt stress. Apart from a metagenomic characterization, we intend to isolate a maximum of different microbes and test their capacity to transfer stress tolerance to crop plants. By different screening protocols with the genetic model plant Arabidopsis, we try to identify stress enhancing microbial strains and understand the underlying molecular mechanisms. The long term goal of DARWIN21 is to transfer the gained knowledge to develop new concepts of sustainable agriculture and re-establish agriculture in arid regions of the world.

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Drought impact on the expression of the plant defence arsenal and fungal aggressiveness in the interaction between *Magnaporthe oryzae* and rice

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It is well-studied that plants with improved tolerance to drought frequently exhibit increased susceptibility to pathogens, suggesting a massive negative cross-talk between the two biological processes (Mittler, 2006). However, little is known about plant resistance against pathogens under drought stress (Prasch and Sonnewald 2013). in addition, there is no report on the molecular strategies pathogens adopt when infecting already-stressed plants. Given the available knowledge on genes involved in resistance (receptors, signalling, defence arsenal...) and aggressiveness (developmental regulators, nutrition, effectors...), we chose the model interaction between rice and *Magnaporthe* to characterize how drought is impacting on plant/pathogen interaction.

We have developed a drought protocol that does not significantly affect plant growth but strongly increases susceptibility (2-fold increase in fungal biomass). Surprisingly, preliminary cytological analysis showed slower fungal progression on the leaf surfaces of drought-stressed plants within first day after inoculation. However, two days after inoculation, the development of the fungus was similar and at three and four days after penetration into the plant cells, the fungal growth was higher in stressed-plants than in un-stressed plants. This increase in fungal growth could be attributed to either reduced resistance of the stressed-plants or increased aggressiveness of the fungus, or both. Preliminary data suggest that the observed increased susceptibility of the plants may rather be a consequence of an increase of aggressiveness of the fungus. RNAseq data are being produced to further explore this hypothesis.

The acquisition of a Type 3 Secretion System as a first step for emergence of novel pathogenic strains of *Xanthomonas*.

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Recently, we isolated strains of *Xanthomonas* that did not induce any symptoms upon inoculation on their hosts of isolation. Among these, some strains do not carry any Type 3 Secretion System (T3SS) or Type 3 Effectors (T3Es), and are thus considered as non-pathogenic. When inoculated on non-host plants tobacco or pepper, these strains induced a hypersensitive response (HR). By tri-parental mating, we conjugated into these strains a cosmid carrying the *hrp* cluster that encodes the T3S and 4 T3Es of the model strain *Xanthomonas campestris Xcc*8004. The transconjugated strains were able to suppress the HR on tobacco and pepper induced by the wild type non-pathogenic strain. We identified the HR suppressors among the 4 T3Es present on the *hrp* cluster of *Xcc* 8004 by screening Tn5 insertions that abolished the suppression ability of the cosmid. We thus could show that both XopF1 and Hpa2 play a role in the suppression of the HR induced by environmental non-pathogenic strains on non-host plants.

Therefore, the acquisition by non-pathogenic strains of the T3S and T3Es via conjugation results in the ability to suppress non-host plant defenses induced by environmental strains, and would thus constitute a first step in the emergence of a novel pathogen.

Comparative screen for suppression of early flg22-triggered immunity in tomato and Arabidopsis identifies functionally redundant RXLR effectors from Phytophthora infestans

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ABSTRACT

Genome sequences of several economically important phytopathogenic oomycetes have revealed the presence of large families of so-called RXLR effectors that are thought to manipulate host cellular activities to the benefit of the pathogen. However, less is known about the molecular mechanisms underlying the modes of action of these effectors *in planta*. Perception of highly conserved pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs), such as flg22, triggers converging signaling pathways recruiting MAP kinase cascades and inducing transcriptional re-programming, yielding a generic anti-microbial response. We used a highly synchronizable, pathogen-free protoplast-based assay to screen a library of 33 *Phytophthora infestans* RXLR effectors (PiRXLRs) for their ability to suppress flg22-triggered defense signaling. Eight effectors, called Suppressors of early flg22-induced Immune response (SFI), significantly reppressed a flg22-depedent reporter gene under control of a typical MAMP-inducible promoter (pFRK1-Luc) in tomato protoplasts. We extended our analysis to Arabidopsis thaliana, a non-host plant species of *P. infestans*. From the aforementioned eight SFI effectors, three appeared to share similar functions in both Arabidopsis and tomato by suppressing transcriptional activation of flg22-induced marker genes. A further three effectors interfere with MAMP signaling at, or upstream of, the MAP kinase cascade in tomato, but not in Arabidopsis. Transient expression of the SFI effectors in *Nicotiana benthamiana* enhances susceptibility to *P. infestans.* This study provides a framework to decipher the molecular mechanisms underlying the manipulation of host MAMP-triggered immunity (MTI) by *P. infestans* and to understand the basis of host versus non-host resistance in plants towards *P. infestans*.

Regulate and be regulated: tight control of plant defence responses by the Arabidopsis transcription factor MYB30

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Plant defence responses are often associated to the development of the so-called hypersensitive response (HR), a form of programmed cell death that confines the pathogen to the inoculation site. The sharp boundary of the HR suggests the existence of efficient mechanisms that control cell death and survival. The transcription factor MYB30 is a positive regulator of Arabidopsis defence and HR responses against bacterial pathogens. MYB30 appears to modulate cell death-related lipid signaling by enhancing the synthesis of sphingolipid-containing very long chain fatty acids after bacterial inoculation.

Plant and animal pathogenic bacteria inject type III effectors (T3Es) into host cells to suppress host immunity and promote successful infection.We have shown that MYB30 is targeted by the bacterial T3E XopD, resulting in suppression of MYB30-mediated plant defences and underlining the crucial role played by MYB30 in the regulation of plant disease resistance. In addition, the activity of MYB30 is tightly controlled inside plant cells through protein-protein interactions and posttranslational modifications. Our recent work identified a protease of the subtilase family (SBT) as a MYB30-interacting partner in yeast. Interestingly, we have shown that the *SBT* transcript is alternatively spliced, leading to the production of two distinct gene products that encode either a secreted (SBT-1) or a nucleocytoplasmic (SBT-2) protein. The specific interaction between MYB30 and SBT-2 was confirmed *in planta* using the FRET-FLIM technique. Importantly, analysis of *sbt* mutant Arabidopsis plants identified SBT as a negative regulator of HR and defence responses to bacterial inoculation. The implications of these findings will be discussed.

The ROAD MOVIE project - Resistance Of Apple against Diseases : Mechanisms Of Virulence and Identification of Effectors

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Abstract

During infection, pathogens produce small secreted proteins (SSP), called effectors, that promote disease. The majority of these effectors interact with host cell processes to suppress the host defenses. Some of them, so-called avirulence factors, trigger immunity in hosts through their recognition by proteins mediating resistance (encoded by R genes). Others will facilitate escape to host recognition and will overcome R genes thanks to virulent alleles existing in the population or by emergence of new mutation at the avirulence locus. However it has been demonstrated that some of these effectors involved in "avirulence" also have pleiotropic effects on the pathogen life cycle. Then, loss-of-function mutations may induce a substantial fitness cost. Polymorphism of these genes is then supposed to be driven by two opposite selective forces: diversifying selection that promotes new variants - functionnal or not-useful for virulence and purifying selection that reflects functional constraints in life cycle. Our hypothesis is that purifying selective pressure should be the main driver of a part of these effectors. Our project thus aims at identifying the effector repertoire of *Venturia inaequalis,* its evolutionary dynamics*,* and at studying the role of several candidate effectors in pathogenicity. *De novo* sequencing of 90 strains, collected on apple and on their wild relatives and differing in their host range or virulence allowed us to study polymorphism of 880 putative effectors. We then selected a subcatalogue of 20 SSP (the top-20 hits in our screen for highly constrained genes) as candidate of avirulence gene. *In planta* gene expression analysis, using RTqPCR and microarray data, shows a significant induction of these conserved SSP at the early stage of plant infection. Several of these 20 SSP are overexpressed only 2 hours after inoculation, during recognition and penetration step. Their functions are presently investigated by mutant KO generation. The loss of these conserved SSP induces an important fitness cost in transformants. GFP tagged protein and heterologous expression are used to assess their sub-cellular localization during interaction. Involvement of theses SSP in the modulation of host defence is also investigated. A subset of effectors, exhibiting highly conserved sequences, will be used to screen for novel resistance (R) genes in apple germplasm. This combined knowledge should enable the proposition of new strategies to build durable resistance towards apple scab.

Are cytokinins new effectors from fungi?

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Cytokinins (CKs) are hormones known to be involved in developmental processes and in regulation of important metabolism pathways in plants. Recently their role in plant pathogen interactions has emerged.

Some bacterial and fungal plant pathogens have been described to be able to secrete CKs. Considering their roles in plant physiology, it has been proposed that producing CK could be an advantage for pathogens to manipulate the basal metabolism of their host. Jiang et *al* (2012) showed that the rice blast fungus, *Magnaporthe oryzae*, produces and secretes some CKs but the role of these fungal-derived hormones is not established. It has been hypothesized that during the earlier stages of infection, CKs could serve as effectors to trigger an uptake of nutrient in infected cells that would favor fungal growth. Unexpectedly, our previous results showed that some CKs can increase rice resistance against this pathogen. Thus the role of CKs as effectors of pathogenicity in the interaction between rice and *M.oryzae* is still unclear.

We identified a putative CK biosynthesis gene in *M.oryzae* that is present in several other pathogenic fungi and created knock-out mutants for this gene. Since a secondary biosynthesic pathway could exist in fungi, we also generated mutants that overexpress a gene that encodes for an enzyme which de-activates most CKs by oxidation, *OsCKX2*. Preliminary results showed that *in vitro*, *M.oryzae* CK mutants displayed a delay in germination. This phenotype could be complemented by exogenous CK application. The pathogenicity of *M.oryzae* mutants affected in CK metabolism will be presented.

Comprehensive Transcriptome Profiling of Root-knot Nematode During Plant Infection

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Root-knot nematodes are obligate endoparasites that maintain a biotrophic relationship with their hosts over a period of several weeks. They infect roots as microscopic vermiform second-stage juveniles (J2) hatched from eggs in the soil. J2s are attracted to the host root, penetrate the root apex and migrate between cells to reach the plant vascular cylinder. To further develop and molt into a pear-shaped female that will release hundreds of eggs on the root surface in a protective gelatinous matrix, J2s need to successfully establish and maintain specialized feeding structures called "giant-cells" from which they withdraw water and nutrients allowing their sedentary biotrophic lifestyle.

This project aims to identify nematode genes specifically involved in plant parasitism with a specific emphasis on genes encoding new secreted effectors of parasitism. Using Illumina technologies, we compared transcriptomes of *Meloidogyne incognita* during its life cycle and identified genes specifically expressed in early parasitic stages *versus* pre-parasitic J2 and eggs stages. We first validated specific expression patterns using quantitative RT-PCR. Genes that were confirmed as overexpressed in parasitic stages were further investigated for their putative role as effectors. Obtained results will be presented.

Immune signalling complexes engaged at the chromatin level and targeted by *Ralstonia* PopP2 effector

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Arabidopsis intracellular TNL (Toll/Interleukin1-Nucleotide Binding-Leucine rich repeat) immune receptors RRS1-R (Resistance to Ralstonia solanacearum 1) and RPS4 (Resistance to Pseudomonas syringae 4) function as a dual resistance system able to recognize two bacterial effectors, AvrRps4 and PopP2, respectively from leaf-infecting *Pseudomonas syringae* and root-colonizing *Ralstonia solanacearum*. RRS1-R contains a C-terminal WRKY DNA-binding motif, characteristic of the zinc-finger class of WKRY plant transcription factors suggesting that activated RRS1-R may directly regulate ETI-related transcriptional changes. PopP2 acetyltransferase activity is necessary for RRS1-R activation. Our results show that PopP2 acetylates RRS1-R, thereby disrupting its physical association with DNA. In this context, we are studying by which mechanism(s) this modification triggers the activation of RPS4/RRS1 immune receptor. We are also determining interactions involving RPS4/RRS1-R, chromatin and chromatin-associated PopP2 targets, by using a combination of 2 different FRET-FLIM approaches aimed at the detection of protein-protein and protein-DNA interactions, respectively. We will report on the characterization of protein complexes engaged at the chromatin level and targeted by PopP2.

Molecular Mechanism of NLR immune receptor activation

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Arabidopsis intracellular TNL (Toll/Interleukin1-Nucleotide Binding-Leucine rich repeat) immunereceptors RRS1-R (Resistance to Ralstonia solanacearum 1) and RPS4 (Resistance to Pseudomonassyringae 4) function as a dual resistance system able to recognize, among other effectors, *Ralstoniasolanacearum* PopP2. RRS1-R contains a WRKY DNA binding domain suggesting that activated RRS1-R may directly regulate ETI-related transcriptional changes. PopP2, a YopJ family member, was shown to physically associate with RRS1-R and to display an acetyltransferase activity requiredfor RRS1-R activation. We present data showing that RRS1-R is a substrate of PopP2 enzymatic activity. PopP2 acetylates a critical lysine residue in the WRKY DNA-binding domain of RRS1-R that inhibits its binding to DNA. This study brings new clues in the deciphering of mechanisms by which TNL activate defense upon effector recognition. In addition, our data reveal a novel mechanism developed by a bacterial type III effector which is using acetylation to inhibit the DNA-binding activity of WRKY transcription factors.

Human Toll Like Receptor 9 deregulation by oncoviruses

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Innate immunity is the first line of cellular defense against a wide range of pathogens. We are armed with a panel of cellular receptors that can sense pathogen associated molecular patterns (PAMPs) and activate the expression of cytokines and chemokines that will fight of the infection. In certain cases pathogens, have evolved to escape immunity, by deregulating innate immune sensing. Our work has described that several oncoviruses can block the expression of a key innate senser called. Toll Like Receptor 9 (TLR9). The aim of this presentation is to summarise and discuss the role of TLR9 in innate sensing in mammals and to share our data demonstrating how several oncoviruses can block its function.

Activation of viral transmission induced by vector action on a host: a general phenomenon?

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We have recently shown that cauliflower mosaic virus (CaMV) forms precisely at the aphid vectors' arrival on its host plant specific transmission morphs. Artificial inhibition and induction of their formation decreases and increases transmission rates, respectively. Such a behavior suggests that CaMV perceives – probable via the plant's sensory systems – the presence of the vector and uses it to control its own transmission. One important question emerging from these results is whether other vector-transmitted viruses also control their transmission by sensing arrival of their vector.

To investigate this question, we started to explore transmission of turnip mosaic virus (TuMV), which is also aphid-vectored, but entirely unrelated to CaMV. Using protoplasts purified from infected plants, we found that calcium channel inhibitors decreased aphid transmission of both viruses. On the other hand, azide treatment increased transmission of CaMV, but not of TuMV. Finally, incubation of protoplasts with the reactive oxygen species (ROS) hydrogen peroxide increased dramatically transmission of TuMV, but not of CaMV. Thus it seems that calcium signaling is involved in transmission activation of both viruses, but ROS signaling only in activation of TuMV transmission. This might indicate that reaction pathways diverge after a common calcium-mediated step.

Taken together, our results indicate that with TuMV another virus is able to react on the presence of the vector on the host plant. This is a first step towards generalization of real-time "vector-sensing" and prompt coordination of transmission by viruses.

Specific expression of candidate effectors of the rust fungus *Melampsora larici-populina* during infection of its two host plants, larch and poplar

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Melampsora larici-populina (Basidiomycete, Pucciniales) is one of the rust fungi responsible for the poplar leaf rust disease. It has a complex macrocyclic and heteroecious life cycle, marked by the production of five different spore forms in two different host plants: larch (sexual reproduction) and poplar (asexual clonal reproduction) [1]. The asexual stage leads to the severe rust epidemics recorded in poplar plantations. As for other rust fungi, the asexual stage has been well covered and characterized, whereas we have almost no knowledge of other stages of the life cycle. Particularly, the capacity of the fungus to infect hosts with distinct taxonomical positions raises questions about the molecular bases underlying the specificity of host-rust interactions. Following the genome sequencing of *M. larici-populina* isolate 98AG31 [2], the secretome annotation has revealed a large repertoire of nearly 1200 genes encoding small secreted proteins [3] and expression profiles of these candidate rust effectors have been defined by oligoarray-based transcriptomics during poplar infection [4].
We present here the transcriptome analysis by RNA-Seq (Illumina) of three fungal structures associated to the infection and reproduction stage on larch: basidia, spermogonia, aecia. These were obtained following an original system for controlled infection in laboratory conditions using the reference isolate 98AG31. Almost 300 millions reads were generated for each condition (three biological replicates) and were used to show specific expression profiles in the larch host. Comparison with the profiles previously obtained in poplar reveals the presence of specific sets of candidate effectors expressed either in each or in both host plants.

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Key-words: Transcriptomics, Pucciniales, host specificity

Characterization of root-knot nematode effectors targeted to the nuclei of giant cells and their plant targets

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Root-knot nematodes are obligate endoparasites that maintain a biotrophic relationship with their hosts over a period of several weeks and induce the differentiation of root cells into specialized multinucleate feeding cells, named giant cells. Nematode effectors synthesized in the oesophageal glands and injected into the plant tissue through the syringe-like stylet play a central role in giant cell ontogenesis. In a search for nematode effectors targeted to the giant cell nuclei, we used bioinformatics and comparative genomics on EST and NGS datasets to identify *Meloidogyne incognita* genes encoding proteins potentially secreted upon the early steps of infection. We identified genes specifically expressed in the oesophageal glands of parasitic juveniles that encode predicted nuclear and secreted proteins. We demonstrated that *M. incognita* injects Mi-EFF1 within the feeding cells and that Mi-EFF1 is targeted into the nuclei of the feeding cells. To identify the manipulated host nuclear functions, we performed a yeast-two –hybrid screen in Arabidopsis and tomato. The functional analyses of the identified plant targets will be presented.

Functional analysis of root-knot nematode virulence genes in rice

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Root-knot nematodes are endo-parasites with a wide host range, encompassing mono- and dicotyledonous plant crops. Successful infection is likely achieved by effector proteins produced in the nematode esophageal gland cells and released in the host plant cells. Elucidating the role of different nematode effectors is a key to understanding the molecular basis of nematode parasitism as well as to developing new nematode control strategies. The aim of this study was to assess the functional role of three esophageal gland cell proteins isolated from a *Meloidogyne incognita* cDNA library. The proteins are expressed all along the infection cycle in rice roots and current experiments using specific antibodies are addressing their subcellular localization. Reverse and forward genetic analyses were conducted to assess the role of the candidate proteins in plant-nematode interactions. We used rice (*Oryza sativa*) to generate transgenic plants expressing the candidate genes full-length cDNAs or artificial micro-RNAs (amiRNAs) able to silence the cognate genes in the nematode. Assessment of nematode growth and development on transgenic plants will allow selecting genes involved in establishing the compatibility with the host plant. Protein sequence analyses revealed a high genetic conservation between isolates from different geographical origins. Data obtained should significantly widen our knowledge of molecular players contributing to nematode pathogenicity, opening new avenues for nematode control strategies in rice and other crops of interest.

Abstract

In the search of *L. maculans* effectors involved in the endophytic colonization of oilseed rape
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Phoma stem canker, caused by the fungus *Leptosphaeria maculans*, is one of the most devastating diseases of oilseed rape. This disease is essentially managed through the breeding and the use of resistant varieties. Specific interactions between avirulence genes and corresponding resistance genes, operating according to the gene-for-gene model, are used for genetics control in the fields. However the fungus may overcome those resistance genes which recognize the fungus only during the first stages of plant infection. In that case *L.maculans* colonizes the plant tissues during a long asymptomatic phase, eventually switching to a necrotrophic stage leading to the damaging crown canker.

To date, little is known concerning this symptomless colonization from leaves to the crown. The fungus may either successfully escape plant defenses leading to the stem canker appearance, or the plant can develop an “adult-stage” resistance and no stem canker appears. We hypothesized that effectors could be involved in this endophytic colonization. A pilot RNAseq project evaluated fungal and plant genes expressed at three stages of the infection (early infection, endophytic colonization and stem necrosis). Interestingly, the fungus expresses in the infected stem a high number of genes encoding Small Secreted Proteins (SSPs). These SSP share characteristic features with the early infection effectors, except for their kinetics of expression. They could be promising candidates as effectors involved in the plant systemic colonization. Sixty effector candidates were identified and twelve of them were chosen for further characterization. Their polymorphism in *L. maculans* populations and their involvement in *L. maculans* pathogenicity are currently assessed.

Infection modes of *Plasmopara halstedii*, the agent of sunflower downy mildew disease and characterization of pathogenicity effectors

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Abstract:

Plasmopara halstedii is an obligate biotroph oomycete causing downy mildew disease on sunflower, an economically important cultivated crop. Disease symptoms observed in fields, plant dwarfism, leaf bleaching, sporulation and production of infertile flowers impair strongly seed yield. *P.halstedii* pathotypes are defined by their divergent virulence profiles in a set of sunflower differential hosts carrying different *Pl* Resistance genes, not yet cloned. Number of pathotypes increased to 16 during the last 25 years in France, concomitantly with the breakdown of *Pl* resistance loci used in fields. Studying disease infection mechanisms and pathogen molecular determinants is a prerequisite for deciphering plant sustainable resistance.

We set up infection conditions on sunflower plants grown in hydroponic cultures that mimic field plant symptoms, to follow life cycle and infection modes of *P. halstedii*. A couple of downy mildew resistant and susceptible near isogenic sunflower lines was infected in these conditions, in order to get easy access to the infected organs including roots. Scanning Electron Microscopy (SEM) and light microscropy were used to investigate *P. halstedii* life cycle *in planta* during incompatible and compatible interactions: penetration in plant tissues, colonization, haustoria production and dissemination structures.

In parallel, sequencing of cDNA from *P.halstedii* spores and sunflower infected tissues was performed in order to identify molecular determinants of pathogenicity. 100 putative RXLR and CRN type effectors potentially expressed in sunflower infected tissues were found by *in silico* approaches. Transient expression in sunflower of selected effectors fused to GFP is currently done to study their functional role *in planta*.

INCIDENCE OF *MELOIDOGYNE INCOGNITA* ESOPHAGEAL PROTEIN EXPRESSION IN HOST PLANTS AND INTERACTION NETWORKS WITH HOST PROTEINS.

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Root-knot nematodes (RKNs) release effector proteins from their esophageal gland cells into host roots tissues. Whether interactions between *M. incognita* proteins (MSPs) and host proteins govern plant susceptibility to the nematode remain to a key question. To gain insight into the function of MSPs in the plant cells, we performed histological analyses of *Nicotiana tabaccum* plants expressing individual selected MSPs and further infected by *M. incognita*. Overexpression of two MSPs induced an accelerated giant cell formation time-course and an increase of nematode infectivity suggesting that these proteins could play a critical role in parasitism success. Sub-cellular localization analyses of these proteins fused to the GFP marker protein in onion cells showed that MSPs are targeted to the nucleus and in the cytosol. In order to check whether these effectors target host proteins, we are currently performing yeast-two hybrid binary interaction assays, first studying possible interactions with Arabidopsis highly connected cellular hub proteins (Mukhtar et al, Science 2011). Data obtained should significantly widen our knowledge about host-nematode interaction and open new strategies for nematode control.

Characterization of aphid saliva proteins that can be involved in host plant specialization

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Majority of herbivorous insects are specialized to one or a few plant species and cannot perform well on the others. However, little is known about the molecular mechanisms that are involved in the insect specialization to the host plant species. The pea aphid, *Acyrthosiphon pisum* forms at least 11 biotypes each of which is specialized to one or a few legume species. Interestingly, each biotype is strongly associated with one or a few facultative bacterial symbiont(s) some of which are known to change the biology of the host aphid.

We aim to understand the mechanisms that are involved in the aphid specialization to the host plants by focusing on aphid saliva protein composition and the effects of facultative symbionts on aphid-plant interactions. We hypothesize that aphid saliva genes that are under diversifying selection (high rates of non-synonymous to synonymous mutation ratio, dN/dS) with biotype specific polymorphisms and/or expression patterns can be the genes that are involved in host specialization.

By using the resequenced genome of 40 aphid lineages spanning 11 biotypes, we have identified the genes that encode candidate saliva proteins that have high dN/dS ratio with biotype specific polymorphisms. Transcriptomics of 8 pea aphid biotypes revealed that about 60% of aphid saliva genes show biotype specific expression patterns. Based on these data, we have initiated functional analyses of the selected genes while improving tools to study the pea aphid-plant interactions at a molecular level.

Mathilde HUTIN

SCREENING OF AFRICAN WILD RICE DIVERSITY REVEALS A NEW SOURCE OF BROAD RESISTANCE AGAINST *X. ORYZAE* PV. *ORYZAE* DUE TO IMPAIRED *SWEET14*-MEDIATED SUSCEPTIBILITY

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Xanthomonas oryzae pv. *oryzae* (*Xoo*) is the causal agent of Bacterial Leaf Blight (BLB) of rice, which was first reported in the 80's in West-Africa. To promote growth *in planta* and xylem colonization, *Xoo* injects type III effectors into the cytoplasm of the host cell. TAL (**T**ranscription **A**ctivator-**L**ike) effectors are important virulence factors that act as transcription factors and activate plant gene expression to the benefit of the pathogen. Among the few known virulence targets of *Xoo* is the susceptibility *S* gene *SWEET14*, which encodes a sucrose efflux transporter. Interestingly, *SWEET14* is targeted at distinct boxes by four TAL effectors, which belong to strains of different lineages and geographic origins. The evolutionary convergence for the induction of *SWEET14* reflects its crucial role as major determinant of rice susceptibility to *Xanthomonas*. We aimed at screening the natural diversity of wild rice to search for potential unresponsive *SWEET14* allelic variants. To that end, the promoter region of about 110 accessions from phylogenetically distant *Oryza* species was sequenced. Polymorphism analysis highlighted a strong conservation of the DNA boxes targeted by the four TAL effectors. Nonetheless, we identified three *O. barthii* accessions carrying a deletion of 18bp which overlaps with the DNA boxes targeted by AvrXa7 and Tal5. We next hypothesized that the deletion should interfere with AvrXa7 and Tal5 binding-activity and their virulence function. As expected, we demonstrate that *O. barthii* accessions carrying the 18bp deletion confer "resistance" against strains of *Xoo* relying on Tal5 and AvrXa7 for virulence, specifically. Finally, inoculation of the "resistant" *O. barthii* accessions with strains of *Xoo* representing diverse geographic origins and lineages, suggests that the new *SWEET14* allele might be useful for broad and sustainable control of BLB worldwide.

Plant Host Responses to Aphids

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Aphids are devastating plant sap-feeding insects. These insects cause direct feeding damage and transmit the majority of plant viruses, resulting in significant yield losses, particularly in staple food crops. Interestingly, most aphid species are restricted to one or few host plants. However, some species, many of which are of agricultural importance, can infest a wide range of plant species. An important observation is that aphids spend a considerable time on non-host plants, where they probe the leaf tissue and secrete saliva, but for unknown reasons are unable to ingest phloem sap. These findings suggest that aphids, like plant pathogens, interact with non-host plants at the molecular level, but potentially are not successful in suppressing plant defenses and/or releasing nutrients. Recent work suggests that aphids, like plant pathogens, secrete effectors into their host plants to manipulate host cell processes and impact the ability to infest plants. Therefore, our project aims to investigate the role of plant cellular processes in determining aphid host range.

We are comparing interactions of economically important aphid species, *Myzus persicae* (green peach aphid) *Myzus cerasi* (black cherry aphid) and *Rhopalosiphum padi* (bird cherry oat aphid) with host and non-host plants. More specifically, we are characterizing host, versus non-host plant responses upon aphid interaction by investigating the activation of plant defences and changes in plant gene expression. Understanding the role of both plant and aphid proteins in different types of interactions will provide us with key insight that are needed to develop novel strategies to control aphid infestations.

Involvement of effectors in arbuscular mycorrhizal symbiosis establishment

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Arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota, form the most widespread mutualistic symbioses with roots of almost 80% of land plants. In order to fulfill their life cycle, AMF have to penetrate into their host and propagate in cortical cells where they develop a fungal structure, called “arbuscule”, where nutrient exchanges between the two organisms take place. In a pre-symbiotic stage, AMF secrete different compounds that activate host cell reprogramming and prepare root colonization. However, in early steps of symbiosis, plant defences are transiently activated. It is now suggested that AMF possess and secrete effector proteins, such as pathogens, able to inactivate immune responses. Moreover, we can speculate that AMF rely on unique molecular mechanisms allowing the colonization of various hosts and succeed biotrophy establishment. By analogy with pathogens, we hypothesize that secreted proteins have a key role in host invasion. To investigate which secreted proteins are important for colonization, we generated RNAseq libraries from *Rhizophagus irregularis*, an AMF, in interaction with different hosts: *Pisum sativum*, *Lunularia cruciata*, *Brachypodium dystachion* and *Medicago truncatula*. Thus, we generated a list of best induced secreted peptides during mycorrhization. We are currently characterizing the functional role of these putative effectors. After validation of the secretion in a heterologous system, we analysed the subcellular localization of the fungal proteins in planta. Preliminary data on overexpression or silencing approach on a selection of candidates will also be presented.

Genomics-based effector mining in *Xanthomonas translucens*

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The Gram-negative bacterium *Xanthomonas translucens* causes diseases on many monocotyledonous plants belonging to the *Poaceae* family, including barley (*Hordeum vulgare*), rye (*Secale cereale*), wheat (*Triticum* spp.) and triticale (*Triticum* x *Secale*). It has also been recorded on some grasses (*Bromus* spp., *Phalaris* spp., *Elymus repens*) and on other *Poaceae*. Bacterial leaf streak of small grain cereals, caused by several pathovars of *X. translucens*, has been reported to occur on all continents performing agriculture. Little quantitative information is available on losses caused by bacterial leaf streak. Importantly, changes in climatic conditions, agricultural practice and trade of non-certified seeds may accelerate the spread of the disease for which no efficient control measures exist in the field.
In order to gain insight into pathogenicity mechanisms, we analysed 19 genome sequences for the presence of a type III secretion system (T3SS) and T3SS-translocated effectors (T3Es). In addition, we used genomic information to predicted new T3Es and tested them by reporter assays. Specifically, we constructed protein fusions consisting of the N-terminal region of candidate T3Es, containing the type III secretion signal, to an N-terminally truncated AvrBs1 reporter lacking its own secretion signal. Constructs were introduced into *Xanthomonas* and assayed on pepper plants expressing the corresponding resistance gene, *Bs1*. Results on T3E repertoires and possible links to pathovars and host range will be presented.

Identification of *Phytophthora capsici* nuclear effectors targets

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Abstract:

Phytophtora capsici is a major disease causing agent of *Solonaceae* and *Cucurbitaceae* crops. During infection, pathogen effectors target various plant cell components in order to successfully overcome defences. In the context of plant microbe interactions, the host nucleus is emerging as a key organelle, since it has a crucial role in plant responses to the environment including biotic stress, and appears to be targeted by multiple effectors. A number of *Phytophthora capsici* nuclear effectors have been identified including CRN and RxLR effector families. Here, we are interested in nuclear CRN and RxLR effectors targets identification. We performed Y2H *N. benthamiana* library screen of CRN_1_719, RxLR_401 and RxLR_466, which were showed to be localised specifically to the host nucleus and nucleolus during infection. Positive clones were selected for further analysis and confirmation. We identified Importin α as a CRN_1_719 putative target and Light Dependent Short Hypocotyl for both RxLR_401 and RxLR_466. Nuclear effector host target identification and characterisation is essential for understanding effector function in the context of immune signalling, host susceptibility and effector translocation into the host nucleus.

Expression profiles of *Mycosphaerella graminicola* effector genes highlight a succession of different infectious strategies during wheat leaves colonization

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Mycosphaerella graminicola causes one of the most damaging diseases of wheat. *M. graminicola* has a large arsenal of secreted proteins expressed during infection that includes Candidate Effectors (CE, n ≥ 171) (do Amaral *et al.* 2012). However, it remains unclear how CEs facilitate the initial symptomless phase, support fungal invasion and trigger the formation of necrotic sporulating lesions. Using the annotated IPO323 reference genome (Goodwin *et al.* 2012), we selected 27 genes encoding small secreted proteins (i.e. MgSSPs) likely specifically expressed during infection according to EST data. We explored the temporal expression of these genes by quantitative RT-PCR during compatible and incompatible interactions. We confirmed that most selected MgSSPs (75%) are over-expressed (10 to 8000 fold) during plant infection compared to mycelium grown *in vitro* and could be classified fined groups into according to their expression profile during infection. Most MsSSPs (75%) have their maximum of expression during the late symptomless phase (7-10 days post-inoculation, dpi), with a dramatic decrease in expression at early stages of lesion formation (≥ 14 dpi). These effectors could be classified in two groups according to their expression profile during incompatible infection. One subgroup correspond to MgSSPs that are not expressed in incompatible interactions. This expression pattern was also observed for the known chitin-binding effector ECP6 required for infection (Marshal *et al.* 2011). Such effectors might be involved in endophytic growth or plant defense suppression during the symptomless phase. Other MgSSPs were as expressed in incompatible and compatible interactions. Such effectors might be involved in epiphytic growth on the leaf surface, since the fungus did not enter into leaf tissues in the interaction studied. Finally few MgSSPs (15 %) have their maximum of expression during the necrotic symptom phase associated with exponential fungal growth. One MgSSP was expressed during the switch to necrotrophy (10-14 dpi), while two others were only expressed during the necrotic phase (14-21 dpi). These effectors might play a role in triggering necrotic symptom, massive leaf colonization or pycnidia formation. The functional analysis of some of these genes is underway to validate their role during leaf colonization and infection.

do Amaral *et al*. (2012), PLoS ONE 7(12):e49904
Goodwin *et al*. (2012) PLoS Genet 7(6): e1002070
Marshall *et al*. (2011), Plant Physiology 156:756

Using SILAC strategy to identify protein effectors in the wheat-Fusarium graminearum pathosystem

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Fusarium head blight (FHB) is a widespread and destructive disease of wheat. Causal agents of FHB mainly target spikes and substantially alter grain yield and quality through the production of harmful mycotoxin. In spite of a number of agronomic strategies, our ability to control FHB spreading is still limited and requires increasing knowledge at the molecular scale. Deciphering the molecular crosstalk that controls plant - *Fusarium spp* interaction is a gate to develop new strategies to sustain plant resistance. To ensure their own development, pathogens use a wide range of effectors able to interfere with the host immune system and the host metabolism. Although protein effectors can be detected in the secretome of the pathogen, their identity and their roles remain poorly documented. To define the repertoire of fungal effectors dynamically produced by *F. graminearum* during infection, we customized the SILAC (Stable Isotope Labeling by Amino acids in Cell culture) approach. We developed specific culture media for the production of labeled mycelium and obtained a Lys $^{15}N^{13}C$ incorporation rate in the mycelium of up to 90%. The analysis of proteins extracted from infected ears with spores produced by this mycelium will allow labeled fungus proteins to be distinguished from the host proteins and the identification of fungal proteins which were produced during infection from non labeled host substrates. We will present the developments of this approach and the first results obtained with ‘omics’ tools to decipher the molecular dialogue that occurs between wheat and *Fusarium graminearum*.

Keywords: FHB disease, *Fusarium graminearum*, fungal effectors, plant susceptibility, SILAC.

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THE *RALSTONIA SOLANACEARUM* SECRETOME: TYPE III EFFECTORS, TYPE III-ASSOCIATED PROTEINS AND THEIR ROLE IN PATHOGENICITY

Ralstonia solanacearum, the causal agent of bacterial wilt, exerts its pathogenicity through more than a hundred secreted proteins, many of them depending directly on the functionality of a type 3 secretion system. To date, only a few type III effectors have been identified as being required for *R. solanacearum* pathogenicity, which probably results from the existence of functional redundancy among a large effector repertoire. In order to identify groups of effectors collectively promoting disease on susceptible hosts, we investigated the role of post-transcriptional regulations in the control of type III secretion in *R. solanacearum* strain GMI1000. We analyzed the secretome of strain GMI1000 as well as mutants for several type III chaperones or type III secretion-associated proteins using mass spectrometry experiments. This analysis revealed specific subsets of effectors differentially secreted in some of the mutant strains. The pathogenicity of these *R. solanacearum* mutants was evaluated on several plants, and interestingly, different host specificities could be identified. Advantages of such a global approach to highlight sets of T3Es potentially required for *R. solanacearum* pathogenicity on hosts belonging to diverse botanical families will be discussed.

Genetic mapping of QTLs associated with cassava bacterial blight resistance in *Manihot esculenta* Crantz.

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Cassava, *Manihot esculenta* Crantz, is one of the most important crops over the world and has a great potential in plant improvement. The develop of a high dense genetic map as a part of a breeding and QTL discovery approach for cassava bacterial blight (CBB) resistance in this species remains to be a challenge and it is imperative focus efforts and use novel molecular strategies in this regard. Single nucleotide polymorphisms (SNPs) were obtained from a segregating population of 137 full sibs to construct a high-density genetic map of cassava, through genotyping by sequencing (GBS). The genotyping data obtained by GBS covers 87% (463.2 Mb) of the current cassava genome and more than 78 thousands SNPs were obtained. The 51.4% of the total set of SNPs correspond to transitions and 48.6% to transversions. The SNPs were classified according to the region in the genome: CDS (Coding DNA Sequence) and non-coding regions (introns, promoters and UTR (Un-translated Regions). A meaningful number of SNPs, 62.6% (49,429), were located in annotated cassava genome regions. After genotyping and filtering, a final set of 2,236 high quality SNPs were selected to construct a linkage map. The map covered 2,147.37 cM distributed in 18 linkage groups and included 1,782 markers with an average distance of 1.32 cM/marker site. For QTL analysis the progeny was tested for resistance to two Xanthomonas axonopodis pv. manihotis (Xam) strains, the causal agent of CBB, at two locations and under greenhouse conditions. The phenotypic evaluation of the resistance trait revealed continuous variation. Based on the QTLs analysis nine major QTLs were detected, explaining between 11 and 21% of phenotypic variance of resistance to *Xam*.

Title : Characterization of small regulatory RNAs involved in the establishment of giant cells induced by parasitic nematodes of genus *Meloidogyne*.

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Plant response against bioagressors involves the activation and/or repression of a large panel of genes. Recent studies have shown the major role of non-coding small RNAs (ncRNAs) in plant gene regulation during biotic interactions. Root knot nematodes of the genus *Meloidogyne* represent a major agronomic problem on a world wild scale. Their parasitic success is linked to their ability to induce, in the infected root, the development of a feeding site composed by few giant cells. The establishment of these hypertrophied, multinucleate and highly metabolically active feeding cells involved a large transcriptional reprogramming. This project proposes to study the regulatory role of ncRNAs during plant - root-knot nematode interaction. A high throughput sequencing strategy of infected *Arabidopsis thaliana* root ncRNAs has been initiated in order to identify differentially expressed ncRNAs during the establishment of the feeding site. We focus our study on one ncRNA family: microRNAs for which the role in plant gene regulation is well known. Micro RNA regulated during the formation of the feeding site will be ranked according to their expression level and those of their targets. Functional analysis of the most interesting miRNAs will be initiated. The localization of their expression will be analyzed using *in situ* hybridization. In addition, studies based on overexpression or inactivation of these microRNAs will be performed to analyze their importance in the parasitic success. Finally, a comparative approach with tomato crop will be developed to identify microRNA regulated during the establishment of the feeding site and conserved among different plants species. These conserved microRNAs could be interesting targets to develop news resistance strategies to fight against root knot nematodes.

Analysis of the genetic diversity of 8 avirulence genes of the blast fungus *Magnaporthe oryzae*.

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Effectors are key elements in the adaptation of pathogens to their hosts. They allow them to use the host's physiology for their development and to inactivate defense responses. Plants possess immune receptors that enable them to recognize effectors and to activate highly efficient resistance responses against adapted pathogens. Such recognized effectors are called avirulence (Avr) effectors or Avr proteins. Frequently, an arms race driven by effector-mediated virulence and effector recognition by the plant host is engaged. This coevolution, between pathogen effectors and plant immune receptors, may leave different kinds of signatures at the molecular level on the underlying genes: increased level of presence/absence polymorphism, increased evolutionary rates, positive selection.

In this work, we analyzed the genetic diversity of 8 Avr effector-coding genes of the blast fungus *Magnaporthe oryzae* in a core collection of 95 isolates. This core collection is representative of the species-wide variability and has been extensively characterized with genetic makers in neutral loci. We determined the presence/absence polymorphism of the 8 *Avr* genes and analyzed their nucleotide polymorphism by sequencing. In parallel, we used the genome data of 8 sequenced *M. oryzae* to search for homologues of the 8 Avr genes. Results confirmed that the evolution of Avr effector-coding genes reflects the arm-race coevolution with their cognate R genes.

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Mr Kroj,

Through this letter, I'd like to express my interest for your EFFECTOM network and my desire to attend to Effectom meeting (8-10 October – Montpellier). Indeed, the knowledge acquisition about effector can allow identifying new resistance sources important for our breeding programs. My lab works on different pathogens (bacteria, virus, fungus, Oomycete and insect) and different species (lettuce, bean, cauliflower, radish, pea and beet) and this kind of event is a good way to take contact for the prospective projects.

Best regards,

Monsimier Bertrand

COMPUTATIONAL IDENTIFICATION AND FUNCTIONAL CHARACTERISATION OF CANDIDATE DNA BINDING EFFECTORS IN *PHYTOPHTHORA*

G. B. Motion, P. R. J. Birch, E. Huitema and S. Jones

In order to cause devastating diseases *Phytophthora* species perturb the host immune system. Like most pathogens, *Phytophthora* secretes a large repertoire of molecules (effectors) during infection to supress immune responses and enable infection. Once inside host cells many effectors localise to the nucleus, where they are thought to modulate host nuclear processes. Microarray analysis on tomato-*P. capsici* timecourse experiments has revealed dramatic transcriptional changes as a consequence of infection, suggesting a role for pathogen effectors in transcriptional reprogramming during infection.

We hypothesise that *Phytophthora* genomes encode effectors that translocate into host nuclei during infection, where they bind DNA and modify gene expression. To test this, we have implemented a chromatin fractionation assay which allows identification of DNA associated proteins. Candidate DNA binding effectors identified from this are now being used for ChIP-Sequencing to identify their target DNA sequences. Computational methods for predicting DNA binding proteins can provide another useful source of candidate selection. However, current prediction algorithms are limited in plants. We therefore aim to improve upon existing DNA binding predictions by making a plant specific prediction model.

Here we present results confirming the success of our species specific DNA binding prediction model which gives improved results compared to an existing model. We are now working on applying this model as a tool for the annotation of DNA binding proteins in plant genomes and the identification of candidate DNA binding effectors. We also show the use of a chromatin fractionation assay which has allowed identification of several DNA associated effectors.

Deciphering plant antiviral PTI pathways and plant virus effectors

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Since its discovery, the concept of PAMP-triggered immunity (PTI) emerged as a key defense mechanism governing host-pathogen interactions. The last 15 years have seen through great advances in the plant pathology field and an extensive list of PRRs, signalling components as well as pathogenic effectors have been identified. To date, no PRR, no PTI signalling cascade, nor effectors have been identified in plant-virus interactions, and the knowledge related to both antiviral PTI responses and viral effectors relies solely on the animal field. Nevertheless, many converging elements suggest that PTI against viruses does also exist in plants. We are currently addressing both questions of plant antiviral PTI mechanisms and plant virus effectors suppressing PTI responses, using the *Arabidopsis thaliana* - *Plum pox virus* (PPV) pathosystem as a model for plant-virus interactions. Preliminary results revealed that some key signalling components from PTI pathways against non-viral pathogens are also involved in Arabidopsis resistance to PPV. In parallel, our experiments seem to indicate that one PPV protein is able to reduce flg22-induced responses in *Nicotiana benthamiana*, suggesting it might function as a viral effector targeting PTI mechanisms. Further analyses are still in progress.

Xanthomonas campestris effectors delivery: where and when?

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For mesophyll-colonizing bacteria, stomata serve as main entry sites in the leaf and represent the first immune barrier to overcome. Xylem-colonizing bacteria gain access to the leaf vasculature using mostly hydathodes, which are water pores situated at the leaf margin. To date, the genetic and the physiology of hydathode's immune responses to pathogens are unknown. *Xanthomonas campestris* pv. *campestris* (*Xcc*) is the bacterial vascular pathogen causing black rot of Brassicaceae which will be used to try and dissect the very early immune responses at the hydathode level. Our current advances towards this goal will be presented.

The rice NB-LRR protein RGA5 recognizes the *Magnaporthe oryzae* effector AVR-Pia by direct binding

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Plant immunity relies on direct or indirect recognition of pathogen effectors by plant resistance (R) proteins. This recognition activates disease-resistance signaling pathways leading to the inhibition of pathogen growth and the induction of a localized programmed cell death called the hypersensitive response (HR). To gain a better understanding of the molecular mechanisms governing effector recognition in plants, we study the translocated effector Avr-Pia from the rice blast fungus *Magnaporthe oryzae* and its recognition by the rice nucleotide-binding and leucine-rich repeat domain (NB-LRR) proteins RGA4 and RGA5. Yeast two-hybrid and co-immunoprecipitation experiments revealed physical interaction of AVR-Pia to an unconventional domain in RGA5 related to the yeast copper chaperone ATX1 (RATX1 domain). This suggests that AVR-Pia recognition occurs by direct binding and that RGA5 acts as an effector receptor. This hypothesis is supported by the finding that a polymorphic site, present in a natural allele of AVR-Pia, abolishes Avr activity and RGA5-binding. A three dimensional structure model of AVR-Pia was generated using nuclear magnetic resonance spectroscopy (NMR) and the polymorphic site was mapped on the model identifying a region which may be important for RGA5-binding and resistance induction. Point mutations were introduced in this region and elsewhere in AVR-Pia by site-directed mutagenesis and analyzed by yeast two hybrid and transient expression in *Nicothiana benthamiana*. By this, six additional amino acids, crucial for interaction with RGA5 and activation of defense responses were identified. They are located either in the previously identified region or in other sites of the molecule suggesting that different parts of AVR-Pia are engaged in physical interaction with RGA5.

Functional analysis of crinkler effectors from the plant pathogenic oomycete *Aphanomyces euteiches*

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Microbial pathogens translocate effectors inside host cells to subvert cellular functions and suppress immune responses. Oomycetes, which are fungal-like eukaryotic microorganisms that cause some of the most destructive plant diseases in the world, secrete several different kind of effector proteins. Two large groups of these effectors are the RXLR and the CRN (Crinkler) proteins. RXLRs and CRNs are modular proteins with conserved N-termini and highly diverse C-terminal effector domains. We recently obtained the genome sequence of the legume root pathogen *Aphanomyces euteiches (ATCC201684, AphanoDBv2.0;https://www.polebio.lrsv.ups-tlse.fr/aphanoDB/)*. This data revealed the absence of RXLR effectors and the presence of over 150 putative CRN effectors in the genome of this pathogen*. Aphanomyces* sp. CRNs are characterized by the presence of an LYLALK translocation motif, and although many CRNs have been identified data on CRN function and targets is still limited. We started the functional analysis of these CRN effectors to gain insights in the virulence mechanisms of *A. euteiches* and to identify possible targets for disease control. We have been able to show that one of the CRN effectors, CRN13, localizes in the plant nucleus where it triggers cell death. Further, we found that the CRN13 ortholog of the fungal amphibian pathogen *Batrachochytrium dendrobaditis* is able to cause a similar response in both plant and amphibian cells. Additionally, we demonstrated that both CRN13s are able to bind DNA *in vitro* and cause DNA damage *in vivo*. Altogether, this work reveals that CRNs produced by plant and animal pathogens bind DNA to interfere with host responses.

Xanthomonas translucens – do TAL effectors contribute to pathogenicity?

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Bacterial leaf streak (BLS) is the most common bacterial disease of small grain cereals, such as wheat, barley, triticale. This disease has been reported at diverse locations worldwide. *Xanthomonas translucens* (*Xt*) is the bacterial agent responsible of BLS. Since BLS of barley and wheat is a seed-borne disease, it poses significant constraints for international germplasm exchange. Several countries list *Xt* as a quarantine organism. Yield losses as high as 40 percent have occurred in the most severely diseased fields in United States, although losses are generally 10 percent or less. Yet, *Xt* did not receive much attention over the last years. It is still not known in detail which infection route the bacteria follow. Therefore, we use fluorescent reporters to study which tissues are affected during the infection process. There is also not much known about pathogenicity factors of *Xt*. To cause disease, most xanthomonads depend on a highly conserved type III secretion system (Hrp system) which translocates type III effectors (T3Es) into target host cells. A specific family of T3Es, called Transcription Activator-Like (TAL) effectors, modulates plant gene expression upon binding to target boxes in the promoter regions of plant genes. By mimicking eukaryotic transcription factors, TAL effectors induce certain plant gene(s) whose activities are required to establish a susceptible state of the host towards infection. In order to find key virulence determinants involved in the interaction with the host plant, systematic knock-out mutageneses of type III secretion system and *tal* genes were performed and mutants were characterized by pathogenicity assays. Several *tal* genes were cloned and sequenced in order to identify new susceptibility genes in barley.

A two genes – for – one gene interaction between *Leptosphaeria maculans* and *Brassica napus*

Auteurs : Yohann Petit, Alexandre Degrave, Michel Meyer, Françoise Blaise, Bénédicte Ollivier, Thierry Rouxel, Isabelle Fudal, Marie-Hélène Balesdent

Leptosphaeria maculans is a hemibiotophic ascomycete which causes stem canker of oilseed rape. That phytopathogenic fungus interacts with its host (*Brassica napus*) according to the gene-for-gene concept. The most economically and environment friendly method of control of stem canker is the genetic control by using host resistance. Single gene resistance is extremely efficient, but races of the pathogen virulent towards a resistance gene can appear in a few years and necessitates continuously new breeding programs. Moreover, specific resistances are rare in oilseed rape, and a lot of efforts are made to find other resistance genes in other Brassica species. To date, 11 interactions were genetically characterized between *L. maculans* avirulence genes and corresponding resistance genes in *Brassica*, and 5 of those avirulence genes were cloned. Recently, the avirulence gene *AvrLm10* which is recognized by the resistance gene *Rlm10* of the black mustard (*Brassica nigra*) has been cloned. *AvrLm10* corresponds in fact to two avirulence genes *AvrLm10_1* and *AvrLm10_2* which are located in the same AT-rich genomic region. They encore for small secreted proteins (SSP), are co-regulated and over-expressed 7 days post-infection. Each of them is necessary but not sufficient to induce resistance towards *Rlm10*. Silencing of one of those genes is sufficient to abolish recognition by *Rlm10.* Silencing by RNA interference of *AvrLm10-1* induces an increase of lesion size on oilseed rape leaves while silencing of *AvrLm10-2* has no major effect on aggressiveness of the fungus. That interaction of two avirulence genes against one resistance gene is therefore different from the classical gene-for-gene concept. It suggests that AvrLm10_1 and AvrLm10_2 could directly interact and / or that they could target the same plant protein. A Y2H screen suggested a direct interaction between AvrLm10-1 and AvrLm10-2. This interaction was confirmed with Bimolecular Fluorescence Complementation (BiFC) experiments. Coimmunoprecipitation experiments are also in progress to confirm this interaction.

A SMALL-SECRETED PROTEIN OF THE POPLAR LEAF RUST FUNGUS TARGETS PLANT CHLOROPLASTS

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Chloroplasts are central organelles that mediate important processes in plants. Pathogens may benefit by subverting chloroplastic functions to their advantage. Indeed, some Type III effectors of plant pathogenic bacteria, e.g. HopN1, HopK1 and AvrRps4 have been reported to target chloroplasts. To date, fungal and oomycete plant pathogen effectors that function in chloroplasts have yet to be described. We report that a 143 amino acid small-secreted protein from the poplar leaf rust fungus *Melampsora larici-populina* targets plant chloroplasts. This protein, termed Chloroplast-Targeted Protein 1 (CTP1), is expressed in haustoria during poplar leaf infection and carries a predicted 80 amino acid chloroplast transit peptide (cTP) downstream of its signal peptide. A CTP1 C-terminal fusion to the Green Fluorescent Protein (GFP) traffics to chloroplasts when it is transiently expressed in the cytosol in *Nicotiana benthamiana* leaf cells. The predicted cTP is cleaved *in planta*, and is sufficient to translocate GFP into chloroplasts. We conclude that CTP1 is a complex modular protein that utilises a cleavable cTP to target chloroplasts. CTP1 is part of a *Melampsora*-specific family of polymorphic small-secreted proteins. Several CTP1 homologs from *M. larici-populina* and from the flax rust fungus *M. lini* also target chloroplasts. We hypothesize that the CTP1 family has evolved recently in *Melampsora* species to manipulate host chloroplasts functions. Ongoing research aimed at elucidating CTP1 function will be presented.

Interaction between avirulence genes *AvrLm3* and *AvrLm7* of the phytopathogenic fungus *Leptosphaeria maculans*

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Leptosphaeria maculans is an ascomycete responsible for phoma stem canker of oilseed rape (*Brassica napus*). The deployment of cultivars with major resistance (*Rlm* genes) is the most effective way to control this disease. We recently found that a negative correlation exists between two avirulence genes *AvrLm3* and *AvrLm7* of the fungus: the presence of a functional allele of *AvrLm7* prevents the isolate harbouring *AvrLm3* to be recognized by *Rlm3*. In the context of current massive deployment of the *Rlm7* resistance in the fields, isolates virulent towards *Rlm7* are selected but all express the previously hidden AvrLm3 phenotype. This interaction offers unprecedented opportunities to design sustainable management strategies to control this pathogen.

With *AvrLm7* having been cloned by Parlange *et al*, (2009), my objective was to clone *AvrLm3* and functionally decipher the interaction between AvrLm3 and AvrLm7. Combining several approaches (RNA-seq, BAC clone sequencing, and *de novo* sequencing of an avirulent isolate), I identified an *AvrLm3* candidate.

In addition, I set up experiments allowing the recovery of few isolates showing virulence towards both *Rlm3* and *Rlm7*. The majority of them possess the same point mutation on the AvrLm7 protein, which had never been observed in natural populations. In this talk I will present data on characteristics of AvrLm3 and preliminary informations on its interaction with AvrLm7.

Huang YJ et al., 2010. European Journal of Plant Pathology 126, 279-291.

Parlange F *et al.*, 2009 Molecular Microbiology 71, 851-863.

Adaptive amino acid changes and the acquisition of a signal peptide in the three genes *Zt80707, Zt89160* and *Zt103264* have contributed to host specialization of the fungal wheat pathogen *Zymoseptoria tritici*

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The ascomycete fungus *Zymoseptoria tritici* (synonym: *Mycosphaerella graminicola)* emerged as a new pathogen of cultivated wheat during crop domestication about 10.500 years ago. *Z. tritici* and closely related species *Z. pseudotritici* and *Z. ardabiliae* are adapted to different hosts and environments. While *Z. tritici* evolved in an agroecosystem and is specialized to wheat, both ancestral species infect a variety of wild grass species. To understand the molecular basis of host specialization in these pathogens we have sequenced complete genomes of the three pathogens. Evolutionary genomic analyses allowed us to identify a set of genes showing strong evidence of positive selection between *Z. tritici* and the closely related sister species. We hypothesize these genes evolved and diverged in response to distinct host traits. None of the genes encode proteins with known function. In this study we focus on three candidate genes *Zt80707, Zt89160* and *Zt103264* and investigate their role in *Z. tritici* during host infection.

Quantitative Real time PCR experiments show that all three genes are expressed only during the infectious phase of the pathogen. Deletion strains for each candidate gene have been created by *Agrobacterium tumefaciens* mediated transformation. Two deletion strains (*Zt80707* & *Zt103264*) showed reduced virulence on wheat. The deletion of the third gene *Zt89160* led to a hypervirulent phenotype suggesting an avirulence function of the gene product. The replacement of *Zt80707* with the homologous gene of *Z. pseudotritici* in *Z. tritici* further reduces the virulence in comparison to the deletion strain. Replacement of the *Z. pseudotritici* homolog of *Zt103264* could only partially restore wild type virulence levels. These results support the importance of adaptive amino acid changes during specialization and speciation of *Z. tritici* and *Z. pseudotritici*. The most intriguing finding is the generation of a signal peptide in the gene *Zt80707* in *Z. tritici* that is absent in the homologous genes of the wild grass pathogens. This suggests a new function of the *Zt80707* protein in *Z. tritici* and

may reveal a species specific trait related to host specialization. Our study confirms that genes involved in host specialization can be identified based on footprints of natural selection.

Negative retrocontrol of the *hrp* genes in the bacterial plant pathogen *Ralstonia solanacearum*

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Ralstonia solanacearum can cause the bacterial wilting disease in plants through the Type III Secretion System (T3SS), through which it injects effector proteins inside the plant cell to change its normal physiology subverting defence responses. The T3SS is encoded by the *hrp* (HR and pathogenicity) genes. Expression of the T3SS is controlled by a regulatory cascade that involves several regulators. The inputs that positively activate the cascade have been well described and include environmental signals as well as plant cell contact. In contrast, no negative regulation of the system has been reported to date. In this work we describe a negative feedback regulation exerted on the master T3SS transcriptional regulator HrpB when bacteria grow in co-culture with *Arabidopsis thaliana* cells. We study if the responsible for this negative feedback regulation is HrpB or HrcC, a T3SS structural protein whose gene is expressed in a transcription unit together with *hrpB*. We will also provide insight of which level of the regulatory cascade is the HrpB\HrcC negative control acting in. We present data on transcriptional levels over time measured in both minimal media and co-culture conditions using a wide array of luminescent reporter strains created in the present study. The new negative regulatory pathway we describe may help to understand how expression of the T3SS system and its related effectors is turned off during the infection process after their rapid initial activation.

Intrincate regulation of the defence-related Arabidopsis MYB30 transcriptional complex through plant proteins and bacterial effectors

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The MYB transcription factor MYB30 was previously identified as a positive regulator of Arabidopsis defense and associated cell death (HR) responses to bacterial pathogens. We previously showed that MYB30 is targeted by the *Xanthomonas* type III effector XopD resulting in suppression of MYB30-mediated plant defences and underlining the crucial role played by MYB30 in the regulation of plant disease resistance. In addition, the activity of MYB30 is tightly controlled by plant cells. In particular, the RING-type E3-ubiquitin ligase protein MIEL1 (MYB30-Interacting E3 Ligase1) interacts with MYB30 in the plant cell nucleus, leading to MYB30 ubiquitination and proteasomal degradation. As a result, MIEL1 negatively regulates MYB30-mediated transcriptional activation and Arabidopsis defence and HR responses. Also, we have recently shown that MYB30 is additionally regulated through its interaction with a protease of the subtilase family. Finally, recent data indicate that XopD is able to interact not only with MYB30 but also with some of its regulatory proteins, which reinforces the idea of effectors as promiscuous proteins able to associate with multiple host components and to affect distinct processes in plant cells. Intriguingly, some of MYB30 regulatory proteins appear to be able to modify XopD accumulation and subnuclear distribution. The existence of separate, nonredundant regulatory mechanisms that temporally and spatially regulate the activation of MYB30-mediated HR and the strategies deployed by bacteria to circumvent these plant responses will be discussed.

Functional studies of putative effectors of *Colletotrichum higginsianum.*

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Colletotrichum higginsianum causes anthracnose disease on cruciferous plants, including *Arabidopsis*. It uses a hemibiotrophic infection strategy, involving formation of several specialized cell types. After melanized appressoria puncture host surfaces, bulbous biotrophic hyphae develop inside living host cells, surrounded by a modified host plasma membrane; finally, the fungus switches to destructive necrotrophy, associated with thin filamentous hyphae. The *C. higginsianum* genome encodes 365 putative secreted effectors (ChECs), of which 97 are expressed *in planta*. Deep-sequencing of the *C. higginsianum* transcriptome during infection (Kleemann *et al.* 2012) and genome-wide expression analysis (O'Connell *et al.* 2012) have revealed a set of plant-induced, appressorium-specific effectors, expressed before host penetration. These proteins were defined as putative effectors on the basis that they have an N-terminal secretion signal and either no homology to proteins outside the genus or similarity to known effectors from other pathogens. 65 candidates were selected for functional analysis based on high expression in appressoria or biotrophic hyphae. This project aims to understand the role of appressorial effectors in the early establishment of infection. The contribution of candidate effectors to fungal virulence and/or avirulence is being investigated by delivering the proteins into *Arabidopsis* leaf cells using the bacterial type III secretion system. For this, the ChECs were cloned into the Effector Detector Vector (EDV) for delivery by *Pseudomonas* species. Clues to effector function may also come from knowing their destination inside host cells. For localization, we use *Agrobacterium tumefaciens* to transiently express effectors as fluorescent protein fusions in *Nicotiana benthamiana* leaf cells. The transient expression *in planta* of 58 ChECs as N-terminal fusions with GFP has revealed different patterns of localisation. Six ChECs are targeted to the plant nucleus, one decorates microtubules and others are targeted to plant organelles. Taken together, these data suggest some effectors are translocated into host cells.

Kleemann et al. 2012 PLoS Pathogens 8: e1002643
O'Connell et al. 2012 Nature Genetics 44: 1060-1067

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Epigenetic hotspots as ecological niches for pathogenicity-related genes?

Leptosphaeria maculans 'brassicae' and *Zymoseptoria tritici* belong to the Dothideomycete class of Fungi, which comprises many devastating plant pathogens. Both species have a hemibiotrophic life style, underlining the involvement of complex regulatory networks. They both present interesting genomic features: *L. maculans* 'brassicae' presents an isochore-shaped genome structure with the alternation of GC-isochores, gene-rich, and AT-isochores, gene-poor but enriched in effector genes that are lowly expressed during axenic culture and highly induced during infection of oilseed rape leaves (Rouxel *et al*., 2011); *Z. tritici*, a wheat pathogen, with eight conditionally dispensable chromosomes (CDCs), is the species with the largest known number of CDCs. These CDCs are twice richer in transposable elements (TEs) than core chromosomes (Goodwin *et al*., 2011). Evidences are accumulating that the location of genes putatively involved in disease establishment, such as effector genes, in genomic areas enriched in TEs is not a random feature. In *L. maculans* 'brassicae', an epigenetic control of the expression of effector genes located in AT-isochores has been demonstrated (Soyer *et al*., 2014). The genomic environment surrounding at least two effector genes, *AvrLm1* and *AvrLm4-7*, is enriched in 'silent' mark of the chromatin, *i.e.* the post-translational histone modification H3K9me3, in axenic cultures. This mark is associated with a repression of gene expression. By silencing expression of two genes involved in the deposition of H3K9me3, expression of effector genes was induced during axenic cultures, *i.e*. at a moment when they are normally silenced. Global transcriptomic analyses in these transformants have demonstrated the existence of hotspots of epigenetic control throughout the genome, not only restricted to AT-isochores. Besides, 74% of the genes over-expressed due to these silencings are naturally over-expressed in the wild type strain *in planta.* This suggests a global epigenetic regulation of genes required for establishment of the infection. This regulatory mechanism would supposedly allows for an efficient regulation of these genes in response to environmental changes. In *Z. tritici*, genes located in CDCs are lowly expressed in axenic culture compared to genes located in core chromosomes (Kellner *et al*., in revision). ChIP-seq analysis in *Z. tritici* has shown that this expression feature of genes in CDCs is consistently associated with an enrichment in 'silent' marks of the chromatin (K. Schotanus, unpublished data) and also that hotspots of epigenetic control are located in discrete foci of the core chromosomes. An epigenomic analysis has been set up in both species to identify areas of the genomes putatively involved in pathogenicity.

Characterization of aphid saliva proteins that can be involved in host plant specialization

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Majority of herbivorous insects are specialized to one or a few plant species and cannot perform well on the others. However, little is known about the molecular mechanisms that are involved in the insect specialization to the host plant species. The pea aphid, *Acyrthosiphon pisum* forms at least 11 biotypes each of which is specialized to one or a few legume species. Interestingly, each biotype is strongly associated with one or a few facultative bacterial symbiont(s) some of which are known to change the biology of the host aphid.

We aim to understand the mechanisms that are involved in the aphid specialization to the host plants by focusing on aphid saliva protein composition and the effects of facultative symbionts on aphid-plant interactions. We hypothesize that aphid saliva genes that are under diversifying selection (high rates of non-synonymous to synonymous mutation ratio, dN/dS) with biotype specific polymorphisms and/or expression patterns can be the genes that are involved in host specialization.

By using the resequenced genome of 40 aphid lineages spanning 11 biotypes, we have identified the genes that encode candidate saliva proteins that have high dN/dS ratio with biotype specific polymorphisms. Transcriptomics of 8 pea aphid biotypes revealed that about 60% of aphid saliva genes show biotype specific expression patterns. Based on these data, we have initiated functional analyses of the selected genes while improving tools to study the pea aphid-plant interactions at a molecular level.

To move between hosts, many plant viruses such as the *Cauliflower mosaic virus* (CaMV) use aphids as vectors. When the insects feed on infected leaves, virus particles already present in the leaf attach to their mouth parts (stylets). This enables the viruses to be transferred to a new host plant. As a prerequisite for CaMV transmission, the viral helper protein P2 acts as a molecular linker and mediates binding of the virus particles to the aphid stylets. P2 is available in infected plant cells in a specific structure that is formed beforehand during CaMV infection. This structure is specialized for transmission and named the transmission body (TB). When inserting its stylets into an infected leaf the aphid triggers ultra-rapid explosion of the TB after massive influx of tubulin As a consequence, P2 is redistributed onto cortical microtubules, together with viral particles (that are simultaneously set free from intracellular storage sites) and forms the so- called mixed networks (MNs). The MNs are the predominant structure from which virus is acquired by aphids; inhibiting their formation reduces drastically transmission rates. Even though the importance of P2MNs for transmission is well established, the P2 motifs and domains involved in formation and functionality of the TB are mostly unknown. We used “Alanine scanning” as a tool to generate a set of P2 mutants. These were analyzed for their capacity to form TBs and induce MNs in infected cells, for interaction with microtubules, and for transmission activity in membrane feeding assays. The results allow to define distinct P2 regions that are presented. In another project, we want to identify elicitors that trigger the TB response. For this, we screen different compounds for their capacity to induce MNs in CaMV-infected protoplasts. Preliminary results confer a role to chitin. In parallel, an *Arabidopsis thaliana* mutant screen has been initiated as a way to dissect the genetic and molecular basis of signal perception and signal transduction pathways involved in the TB response. The latest results will be presented.

Characterization of root-knot nematode effectors targeted to the nuclei of giant cells and their plant targets

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Root-knot nematodes are obligate endoparasites that maintain a biotrophic relationship with their hosts over a period of several weeks and induce the differentiation of root cells into specialized multinucleate feeding cells, named giant cells. Nematode effectors synthesized in the oesophageal glands and injected into the plant tissue through the syringe-like stylet play a central role in giant cell ontogenesis. In a search for nematode effectors targeted to the giant cell nuclei, we used bioinformatics and comparative genomics on EST and NGS datasets to identify *Meloidogyne incognita* genes encoding proteins potentially secreted upon the early steps of infection. We identified genes specifically expressed in the oesophageal glands of parasitic juveniles that encode predicted nuclear and secreted proteins. We demonstrated that *M. incognita* injects Mi-EFF1 within the feeding cells and that Mi-EFF1 is targeted into the nuclei of the feeding cells. **To identify the manipulated host nuclear functions, we performed a yeast-two –hybrid screen in Arabidopsis and tomato. The functional analyses of the identified plant targets identified will be presented.**

The Effector Protein MiSSP7 of the Mutualistic Ectomycorrhizal Fungus *Laccaria bicolor* Interacts with *Populus* JAZ Proteins

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Ectomycorrhizal (ECM) fungi, such as *Laccaria bicolor*, support forest growth and sustainability by providing growth limiting nutrients to their plant host through a mutualistic symbiotic relationship with host roots. We have previously shown that the effector protein MiSSP7 encoded by L. bicolor is necessary for the establishment of symbiosis with host trees, although the mechanistic reasoning behind this role was unknown. We demonstrate here that MiSSP7 interacts with the *Populus* host protein PtJAZ6, a putative negative regulator of jasmonic acid (JA) signaling in *Populus*. As with other characterized JAZ proteins, PtJAZ6 also interacts with PtCOI1 in the presence of the JA mimic coronatine and PtJAZ6 is degraded in plant tissues after JA treatment. The association between MiSSP7 and PtJAZ6 is able to protect PtJAZ6 from this JA induced degradation. Further, MiSSP7 is able to block, or mitigate, the impact of JA on *L. bicolor* colonization of host roots. We show that the loss of MiSSP7 production by *L. bicolor* can be complemented by transgenically varying the transcription of PtJAZ6 or through inhibition of JA signaling. We conclude that *L. bicolor*, in contrast to arbuscular mycorrhizal fungi and biotrophic pathogens, promotes mutualism by blocking JA action through the interaction of MiSSP7 with PtJAZ6.

Arabidopsis RRS1-R immune receptor senses PopP2 acetyltransferase activity

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Arabidopsis intracellular TNL (Toll/Interleukin1-Nucleotide Binding-Leucine rich repeat) immunereceptors RRS1-R (Resistance to Ralstonia solanacearum 1) and RPS4 (Resistance to Pseudomonassyringae 4) function as a dual resistance system able to recognize, among other effectors, *Ralstoniasolanacearum* PopP2. RRS1-R contains a WRKY DNA binding domain suggesting that activated RRS1-R may directly regulate ETI-related transcriptional changes. PopP2, a YopJ family member, was shown to physically associate with RRS1-R and to display an acetyltransferase activity requiredfor RRS1-R activation. We present data showing that RRS1-R is a substrate of PopP2 enzymatic activity. PopP2 acetylates a critical lysine residue in the WRKY DNA-binding domain of RRS1-R that inhibits its binding to DNA. This study brings new clues in the deciphering of mechanisms by which TNL activate defense upon effector recognition. In addition, our data reveal a novel mechanism developed by a bacterial type III effector which is using acetylation to inhibit the DNA-binding activity of WRKY transcription factors.

Human Toll Like Receptor 9 deregulation by oncoviruses

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Innate immunity is the first line of cellular defense against a wide range of pathogens. We are armed with a panel of cellular receptors that can sense pathogen associated molecular patterns (PAMPs) and activate the expression of cytokines and chemokines that will fight of the infection. In certain cases pathogens, have evolved to escape immunity, by deregulating innate immune sensing. Our work has described that several oncoviruses can block the expression of a key innate senser called. Toll Like Receptor 9 (TLR9). The aim of this presentation is to summarise and discuss the role of TLR9 in innate sensing in mammals and to share our data demonstrating how several oncoviruses can block its function.

"Facing and understanding the ever-increasing effector arsenal of *Rhizophagus irregularis* in the arbuscular mycorrhiza symbiosis"

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Mutualistic associations are defined by a beneficial outcome for both interaction partners. In the case of mycorrhizal fungi, the plant is provided with soil-limited mineral nutrients while the fungus receives photoassimilates from the plant. The establishment and maintenance of such a fungal biotrophic lifestyle is accompanied by communication with the plant. However the plant basal immunity universally reacts against microbial intruders regardless of whether they are mutualistic or pathogenic organisms. This happens upon recognition of reactive MAMPs (microbe-associated molecular patterns) that are shared by both mutualistic as well as pathogenic fungi (Jacobs *et al*., 2011, Kloppholz *et al*., 2011).

At least since the discovery of SP7 and MiSSP7 in 2011, two effector proteins from the endomycorrhizal fungus *Rhizophagus irregularis* and the ectomycorrhizal fungus *Laccaria bicolor*, respectively (Kloppholz *et al*., 2011, Plett *et al*., 2011) it is accepted that mycorrhizal just like pathogenic fungi use effector-derived strategies to alter host biology in order to overcome the plant immunity and facilitate colonization. Thanks to the release of the genome of the arbuscular mycorrhiza (AM) fungus *R. irregularis* (Tisserant *et al*., 2013, Lin *et al*., 2014) it is now possible to identify new effector candidates using *in silico* analyses. Among other promising candidates, two distinct families, the SP-protein family with the already characterized member SP7, and the Crinkler-like family with members that share homologies to the well described effectors from plant pathogenic oomycete species (Torto *et al*., 2003, Gaulin *et al*., 2008, Haas *et al*., 2009, Schornack *et al*., 2010, Stam *et al*., 2013) can be identified. The aim of this study is to assign symbiotic functions to these new effector candidates during the AM-symbiosis and to understand their mode of action. Quantitative real-time PCR analysis confirmed the transcription of the putative effectors in mycorrhizal roots. Effector-GFP fusion constructs expressed *in planta* revealed localizations in specific cytoplasmic compartments such as the plant nucleus, or in the apoplast, indicating different plant targets of the effector candidates. Further *in planta* expression assays are in progress and first results are indicative of some candidates being able to alter host physiology significantly.