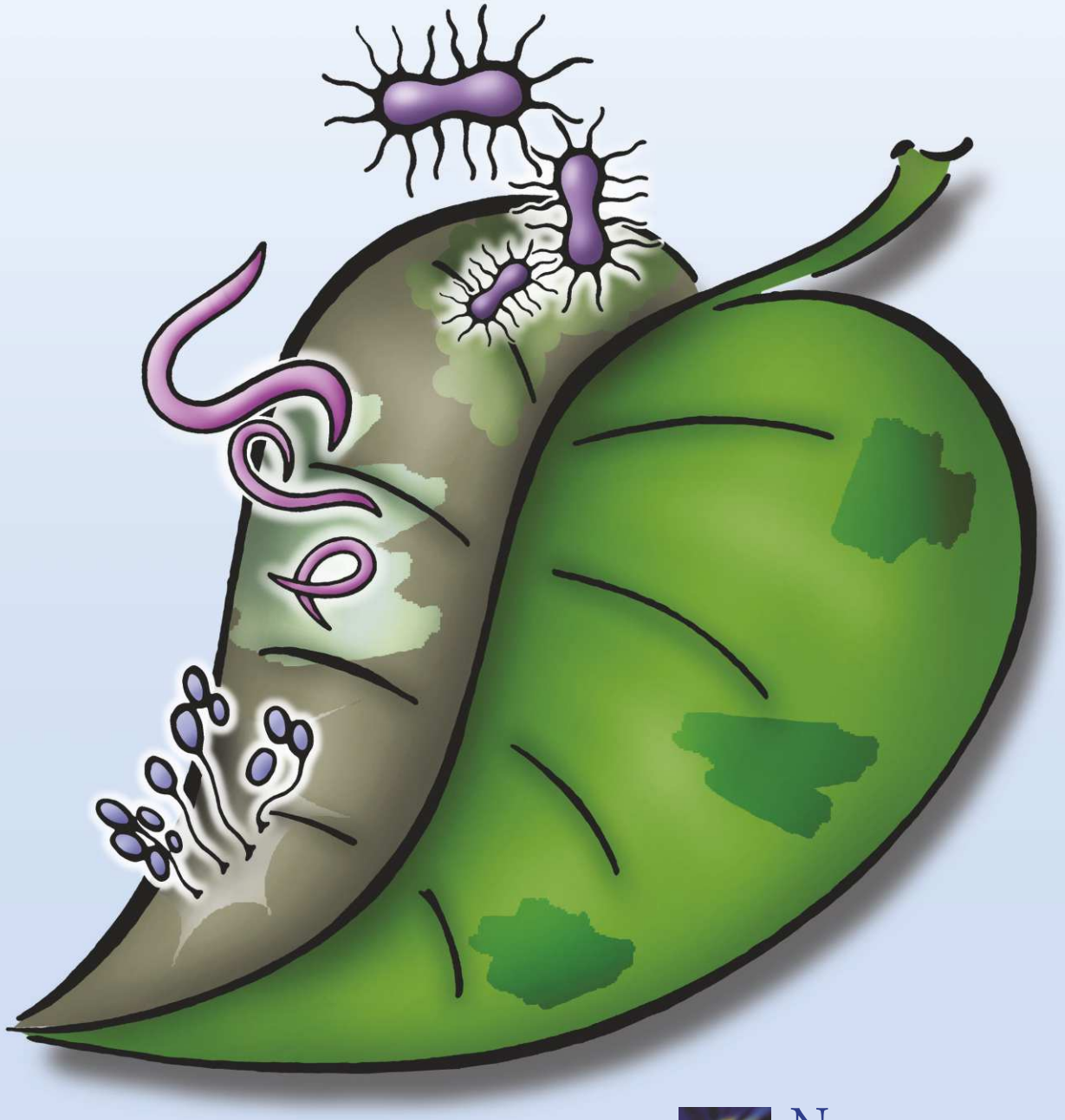
30th New Phytologist Symposium

Immunomodulation by plant-associated organisms

16–19 September 2012
Fallen Leaf Lake, California, USA

Programme, abstracts and participants



New Phytologist

30th New Phytologist Symposium

Immunomodulation by plant-associated organisms

Fallen Leaf Lake, California, USA

Scientific Organizing Committee

Sophien Kamoun *(The Sainsbury Laboratory, UK)*
Brian Staskawicz *(University of California, Berkeley, USA)*

New Phytologist Organization

Helen Pinfield-Wells *(Deputy Managing Editor)*
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Acknowledgements

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New Phytologist Trust

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Programme, abstracts and participant list compiled by Jill Brooke
'Immunomodulation by plant-associated organisms' illustration by A.P.P.S., Lancaster, UK

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Information for Delegates

Meeting Location and Contact
The 30th New Phytologist Symposium will be held at Stanford Sierra Conference Centre, Fallen Leaf Lake, California, USA, 16–19 September 2012. Physical Address: 130 Fallen Leaf Road, Fallen Leaf, CA 96151, USA. Cell phone reception can be poor at Fallen Leaf Lake; to ensure you can be contacted in an emergency please provide the following telephone number to your relatives +1 (530) 541-1244 and any urgent messages will be passed on to you.

All talks will be given in the Cathedral Room. Posters will be displayed in the Angora Room.

Catering
Breakfast will be served from 07:00–08:00 in the Dining room.
Coffee breaks will be served on the Fountain Deck outside the Angora Room in the morning.
Lunch and Dinner will be served in the Dining Room. On Wednesday 19 September lunch will be available from a Hiker Bar which will be open during breakfast and throughout the morning. Delegates can select snacks/drinks and prepare sandwiches at this Bar.
During the **breaks** on Monday and Tuesday evening refreshments will be served in the Cathedral Foyer. Each evening after sessions end the Fountain (bar) will be open.

Accommodation
Check-in is from 15:00 on Sunday 16 September. Please note you must check-out of your room by 10:00 on the morning of Wednesday 19 September. There will be space allocated where you can leave your baggage until departure.

Posters
If you have submitted a poster abstract to share your research with the community this should be A0 in size and portrait in orientation. Please display your poster as soon as possible on the 16 September, on the numbered board which corresponds with the number your poster abstract has been allocated in this abstract book. Please remove all posters by 13:30 on Wednesday 19 September.

Posters will be open for viewing throughout the symposium and will be located in the Angora Room. A dedicated poster session will be held on Monday from 16:00; please stand by your poster at this time and remember there will be prizes!

Map
A map showing the location of the main lodge and cabins is at the back of this book.

Internet Access
Fallen Leaf Lake offers free internet access throughout. The access code is funonthelake

Altitude
The altitude of Fallen Leaf Lake is 6,377 feet (1944 metres). This elevation does not usually cause altitude sickness, but to avoid problems such as dry sinuses and shortness of breath, particularly if you plan on hiking any of the surrounding peaks, drink plenty of water and cut down on your exercise for the first few days of your stay. You should also note that alcohol consumption can exacerbate the effects of altitude.

Information for Delegates

Free time

The boat dock will be open from 13:00−16:00 on Monday and 13:45−17:00 on Tuesday weather permitting. There are also hiking trails, tennis, sand volleyball, table tennis, horseshoe and basketball courts onsite. The onsite shops (Fountain) will also be open. More information will be available from the front desk and can be found at http://www.stanfordsierra.com/recreation

Meeting Programme

Sunday 16 September

17.00–18:30	Registration
18:30–18:45	Welcome from the organisers
18:45–19:00	**Introduction: Origin and history of the Fallen Leaf conferences** *Clarence Kado*
19:00–20:00	**Keynote Presentation: Type III secretion machines: bacterial devices for protein delivery into eukaryotic cells** *Jorge Galán*
20:00	Dinner

Monday 17 September

8.00–8.05	Announcements
Session 1: ***Chair:***	**Pathogenomics** ***Brian Staskawicz***
8:05–8:50	**High throughput genomic sequencing of *Xanthomonas* field strains identifies core effectors to target for durable resistance in cassava and tomato** *Brian Staskawicz* (*Talk and Session Overview*)
8:50–9:25	**Dissecting the co-evolutionary arms race between bacterial effectors and the plant immune system** *David Guttman*
9:25–9:45	Selected talk: *Richard Michelmore* on P58
9:45 –10:15	Coffee break
10:15–10:50	**Transferred effectors of smut fungi** *Regine Kahmann*
10:50–11:25	**Toward understanding *Magnaporthe oryzae* effector functions** *Ryohei Terauchi*
11:25–11:45	Selected talk: *Dinesh Kumar* on P70
11:45–12:00	Discussion and questions from the session
12:00–13:00	Lunch

Afternoon free for activities and viewing posters

16:00–18:00	Poster session and reception
18:00–19:00	Dinner

Meeting Programme

Session 2: ***Chair:***	**Effector secretion and trafficking into host cells** ***Peter Dodds***
19:00–19:45	**Recognition of rust effectors in plant innate immunity** *Peter Dodds* (*Talk and Session Overview*)
19:45–20:20	**Towards understanding mechanisms for targeted secretion of rice blast effectors** *Barbara Valent*
20:20–20:35	Break
20:35–21:10	**Investigating effector delivery by the rice blast fungus *Magnaporthe oryzae*** *Nick Talbot*
21:10–21:45	**Translocation of oomycete RxLR-proteins** *Pieter van West*
21:45–22:00	Discussion and questions from the session

Tuesday 18 September

8:00–8:05	Announcements
Session 3: ***Chair:***	**Induction and suppression of host immunity by microbes** ***Sophien Kamoun***
8:05–8:50	**Modulation of plant immunity by oomycete effectors** *Sophien Kamoun* (*Talk and Session Overview*)
8:50–9:25	***Xanthomonas* AvrBsT targets a microtubule-associated protein involved in immunity** *Mary Beth Mudgett*
9:25–9:45	Selected talk: *Susana Rivas* on P54
9:45–10:20	**Harnessing TAL effector-DNA targeting to understand and prevent plant diseases caused by *Xanthomonas*** *Adam Bogdanove*
10:20–10:50	Coffee break
10:50–11:25	**Multiple targeting of receptor kinase-mediated immunity by bacterial effectors** *Cyril Zipfel*
11:25–12:00	**Effector-induced modification of plant immune signaling** *Gitta Coaker*
12:00–12:35	**Probing jasmonate receptor signaling using small-molecule effector coronatine** *Sheng Yang He*

Meeting Programme

12:35–12:45	Discussion and questions from the session
12:45–13:45	Lunch

Afternoon free for activities and viewing posters

18:00–19:30	Dinner
Session 4: ***Chair:***	**Structural biology of microbial effectors and their targets** ***Jeff Dangl***
19:30–20:15	**Plant immune system function and its battle with pathogen effectors** *Jeff Dangl* (*Talk and Session Overview*)
20:15–20:50	**Protein structures at the interface between type III effectors and the plant immune system** *Greg Martin*
20:50–21:05	Break
21:05–21:40	**Structural insights into TIR domain and effector function in effector-triggered immunity in flax and Arabidopsis** *Bostjan Kobe*
21:40–22:15	**Structure-led studies of plant pathogenic effector proteins** *Mark Banfield*
22:15 –22:30	Discussion and questions from the session

Wednesday 19 September

8:00–8:05	Announcements
Session 5: ***Chair:***	**Emerging systems** ***Francis Martin***
8:05–8:50	**The mutualistic fungus *L. bicolor* uses the effector protein MiSSP7 to alter host jasmonate signaling** *Francis Martin* (*Talk and session overview*)
8:50–9:25	**Plant cell reprogramming during arbuscular mycorrhizal symbiosis** *Natalia Requena*
9:25–9:45	Selected talk: *Gunther Doehlmann* on P59
9:45–10:20	**Functional characterization of effector proteins that modulate plant-insect interactions** *Saskia Hogenhout*
10:20–10:50	Coffee break

Meeting Programme

10:50–11:25	**Cyst nematode effectors: localization, host targets, and functions in plant parasitism** *Melissa Mitchum*
11:25–12:00	**Investigating virulence effectors in the poplar-poplar rust pathosystem** *Sébastien Duplessis*
12:00–12:35	**How oomycete pathogens of Arabidopsis cause or fail to cause disease** *Jonathan Jones*
12:35–13:10	**Hunting for the witch's effectors: Genomic and transcriptomic analyses of the parasitic *Orobanchaceae* plants** *Ken Shirasu*
13:10–13:20	Discussion from the session and close of the meeting

A Hiker Bar will be open during breakfast and throughout the morning on Wednesday 19 September. Delegates can prepare their own lunch for eating after the close of the meeting.

Speaker Abstracts

Introduction

Origin and history of the Fallen Leaf conferences

CLARENCE I. KADO **18:45–19:00**

cikado@ucdavis.edu

Department of Plant Pathology, University of California, Davis, California, USA

The well-known series of plant pathology conferences held at Fallen Leaf Lake were initiated in 1985 by Clarence I. Kado. It was identified that there was a need for an international forum focused on new developments in plant pathology and related fields of research, particularly to address molecular, genetic and evolutionary issues of plant pathogenic and fastidious bacteria interacting with their hosts. Lake Tahoe was a natural choice with a serene environment for providing opportunities for close interactions between young promising scientists and established senior authorities.
This venue was selected through discussions with the late John F. Fulkerson, Chief Scientist of the Cooperative State Research Service of the U.S. Department of Agriculture. CSRS provided the initial funding that launched the Fallen Leaf Lake Conference series. Other agencies followed such as the National Science Foundation, National Institutes of Health, USDA Agricultural Research Service, the European Molecular Biology Organization, the University of California Statewide Biotechnology and Education Program, and the University of California, Davis, and numerous corporate sponsors such as E. I. Dupont de Nemours & Company, Campbell Institute of Research and Technology, Monsanto Company, Ciba-Geigy, Toyota, Merck, Pharmacia, Chevron Chemical, Blackwell Scientific in continued funding of the conference series. Furthermore, complementary fine wines were provided by local wineries such as Stags Leap of Napa Valley and Chateau St. Michel of Washington. The Fallen Leaf Lake Conferences began with the focus on specific genera of plant pathogenic bacteria such as the genus *Xanthomonas*, *Erwinia*, *Agrobacterium,* and *Pseudomonas* in 1985, 1986, 1987 and 1988. Subsequent conferences addressed broader topics such as on the molecular mechanisms of virulence, plasmid biology, biological control mechanisms, horizontal gene transfer and bacterial secretion systems. It is gratifying to see that it continues with broader topics that have drawn many internationally recognized scientists and has encouraged and stimulated new young scientists to pursue their areas of interest. The New Phytologist Symposium at Fallen Leaf Lake promotes this educational and scientific objective.

Keynote Presentation

Type III secretion machines: bacterial devices for protein delivery into eukaryotic cells

JORGE E. GALÁN **19:00–20:00**

jorge.galan@yale.edu

Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT06536, USA

One of the most exciting developments in the field of bacterial pathogenesis during the last two decades has been the discovery that bacterial pathogens utilize specialized Nano machines to deliver bacterial proteins into eukaryotic cells with the capacity modulate a cellular functions for the pathogen's benefit. This strategy is widespread in nature since the presence of this type of machines has been detected in many bacteria pathogenic or symbiotic for animals, plants, or insects. One of these types of machines is known as the Type III Secretion System. I will discuss the structure and function of this machine, with special emphasis on the assembly and function of its most critical component, a subcellular structure known as 'the needle complex'. I will also discuss the mechanisms by which this machine selects substrates in the appropriate order for delivery into target cells.

Session 1: Pathogenomics
Chair: Brian Staskawicz

High throughput genomic sequencing of *Xanthomonas* field strains identifies core effectors to target for durable resistance in cassava and tomato

S1.1

REBECCA BART, MOLLY SHARLACH, MEGAN COHN, ALLISON SWARTZ, MIKEL SHYBUT, ANDREW KASSEN, ANNALISE PETRIELLO, DOUGLAS DAHLBECK, JEFFREY JONES, BRIAN J. STASKAWICZ

8:05–8:50

stask@berkeley.edu

Department of Plant and Microbial Biology, University of California-Berkeley, Berkeley, CA 94720, USA

Next generation Illumina sequencing has been employed to determine the draft genomes of multiple strains of *Xanthomonas axonopodis* pv. *manihotis, X. perforans* and *X. euvesicatoria.* These genomes have been assembled and core type three effectors have been computationally identified. Data will be presented on the phylogenetic relatedness of these strains as deduced from whole genome SNP analysis. Notably, our approaches represent a dramatic increase in the speed in which core effectors can be identified on a population level. We are currently using site-directed gene replacement to evaluate the role of these effectors in virulence. Furthermore, the identification of core effectors will allow us to employ these effectors as molecular probes to identify cognate resistance genes in both host and non-host plants. Our progress in engineering durable resistance in cassava and tomato will be presented.

Dissecting the co-evolutionary arms race between bacterial effectors and the plant immune system

S1.2

AMY HUEI-YI LEE, JENNIFER D. LEWIS, BRENDEN HURLEY, DARRELL DESVEAUX, DAVID S. GUTTMAN

8:50–9:25

david.guttman@utoronto.ca

Department of Cell & Systems Biology, University of Toronto, Toronto, Ontario, Canada

The YopJ/HopZ family of type III secreted effector proteins is evolutionarily diverse and widely distributed among both plant and animal pathogens. We have previously shown that the family diversified in the plant pathogenic bacterium *Pseudomonas syringae* via both mutational processes during vertical descent from the ancestral *P. syringae,* as well as through horizontal transfer from ecologically similar pathogens into five distinct allele groups. We also have shown that the most ancestral allele (HopZ1a) is consistently recognized by the plant resistance protein, ZAR1, and that this interaction induces ETI. Here we identify two virulence targets of HopZ1. We first show that HopZ1a directly interacts with the plant microtubule network, and demonstrate that it is an acetyltransferase that acetylates itself and tubulin. The conserved autoacetylation site plays a critical role in both the avirulence and virulence function of HopZ1a. Furthermore, HopZ1a requires its acetyltransferase activity to disrupt the *Arabidopsis* microtubule network and the secretory pathway as well as to suppress cell wall-mediated defense. We also demonstrate that HopZ1a directly interacts with and acetylates a previously uncharacterized protein kinase (ZED1), which is encoded within a tandemly arrayed family of related kinases, and which also directly interacts with and is required for ZAR1 activation.

Transferred effectors of smut fungi

S1.3

SHIGEYUKI TANAKA, ANUPAMA GHOSH, ARMIN DJAMEI, REGINE KAHMANN

10:15–10:50

kahmann@mpi-marburg.mpg.de

Max Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany

The fungus *Ustilago maydis* is a biotrophic plant pathogen infecting maize. The most prominent symptoms are large plant tumors in which the fungus proliferates. During host colonization *U. maydis* establishes an extended interaction zone in which fungal hyphae are completely encased by the host plasma membrane. Interaction with the plant is largely determined by protein effectors that are conventionally secreted and exert their function either in the interaction zone or are taken up by host cells and reprogram host responses. Many of these effectors are novel, exist only in related smut fungi and locate to clusters in the genome. In my presentation I will concentrate on transferred effectors, their site of action and function after uptake. In addition I will describe how the transferred chorismate mutase Cmu1 can be used to assay translocation.

Toward understanding *Magnaporthe oryzae* effector functions

S1.4

RYOHEI TERAUCHI

10:50–11:25

terauchi@ibrc.or.jp

Iwate Biotechnology Research Center, Kitakami, Iwate, 024-0003, Japan

Rice blast caused by the ascomycete fungus *Magnaporthe oryzae* is the most devastating disease of rice worldwide; therefore understanding of the molecular mechanisms of *Magnaporthe*-rice interactions is important to devise efficient control of the disease. Using *M. oryzae* whole genome sequence information and association genetics approach, we isolated genes for three *AVR*s, *AVR-Pia*, *AVR-Pii* and *AVR-Pik* as well as other effector candidates. All three *AVR*s were shown to be delivered to rice cells. Using biochemical approaches, we are trying to elucidate their effector functions. In this paper, I show our recent findings on their interactions with rice factors including R-proteins.

Session 2: Effector secretion and trafficking into host cells
Chair: Peter Dodds

Recognition of rust effectors in plant innate immunity **S2.1**

PETER DODDS[1], JEFF ELLIS[1], MAUD BERNOUX[1], MICHAEL RAVENSDALE[1], BOSTJAN KOBE[2], SIMON WILLIAMS[2], THOMAS VE[2], ADRIENNE HARDHAM[3], DAVID JONES[3], ANN-MAREE CATANZARITI[3], MARYAM RAFIQI[3], MARKUS KOECK[1], WENJIE WU[3]

19:00–19:45

peter.dodds@csiro.au

[1]CSIRO Plant Industry, Canberra, Australia; [2] School of Chemistry and Molecular Biosciences, University of Queensland, Australia; [3] Research School of Biology, Australian National University, Australia

Rust fungi cause economically important diseases of cereal crops worldwide. We have been studying how the plant immune system can recognise and respond to these pathogens in order to develop novel disease control strategies. Rusts are obligate parasites of plants, and have evolved an intimate cellular association with their hosts. They produce a specialised infection structure called the haustorium which directly penetrates an infected cell and is the main site of nutrient extraction for the fungus. A suite of disease effector proteins are secreted from haustoria and enter the host cells where they may allow the rust to commandeer host cell biology. It is these translocated effector proteins that are recognised by host immune receptors, known as resistance (R) proteins. We are exploring the structure and function of host-translocated effectors, their recognition by host immune receptors, and the receptor signalling activation process, which offers the opportunity to experimentally engineer new recognition capacities.

Towards understanding mechanisms for targeted secretion of rice blast effectors

S2.2

BARBARA VALENT[1], MARTHA C. GIRALDO[1], CHANG HYUN KHANG[1,3], MIHWA YI[1], MELINDA DALBY[1], YASIN DAGDAS[2], YOGESH K. GUPTA[2], NICHOLAS J. TALBOT[2]

bvalent@ksu.edu

[1]Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA; [2]School of Biosciences, University of Exeter, Exeter, EX4 4QD, UK; [3]Current Address: Department of Plant Biology, University of Georgia, Athens, Georgia 30602, USA

19:45–20:20

During biotrophic invasion, *Magnaporthe oryzae* secretes cytoplasmic effectors, which preferentially accumulate in biotrophic interfacial complexes (BICs) and are translocated into the cytoplasm of the rice cells, and apoplastic effectors, which remain in the extracellular space between the fungal cell wall and the rice plasma membrane. BICs localize in front of the tips of filamentous hyphae that enter rice cells, and remain sub-apically beside the first bulbous invasive hyphal cells after hyphal differentiation. In contrast, secreted apoplastic effectors uniformly outline the entire bulbous invasive hypha. We have determined that cytoplasmic effector genes are highly up-regulated in the BIC-associated cells at early invasion stages, and that effector promoters play the major role in determining preferential BIC localization of cytoplasmic effectors. Subapical BIC-associated hyphal cells continue to express protein secretion machinery components while invasive hyphae grow elsewhere in the host cell. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A treatment blocked secretion of apoplastic effectors, but not secretion of cytoplasmic effectors. Pathogen mutants that fail to express exocyst complex components or a t-SNARE are defective in secretion of cytoplasmic effectors. Our data suggest that *M. oryzae* possesses distinct secretory mechanisms for targeting cytoplasmic and apoplastic effectors during rice invasion.

Investigating effector delivery by the rice blast fungus *Magnaporthe oryzae*

S2.3

YOGESH GUPTA, MARTHA GIRALDO, YASIN F. DAGDAS, THOMAS A. MENTLAK, LAUREN S. RYDER, MICHAEL J. KERSHAW, BARBARA VALENT, NICHOLAS J. TALBOT

20:35–21:10

n.j.talbot@exeter.ac.uk

School of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter EX4 4QD, UK

Magnaporthe oryzae is the causal agent of rice blast, one of the most devastating diseases of cultivated rice. During plant infection *M. oryzae* develops a differentiated infection structure called an appressorium. This unicellular, dome-shaped structure generates cellular turgor that is translated into mechanical force to cause rupture of the rice cuticle and entry into plant tissue. The fungus initially forms a narrow penetration hypha inside the rice cell, which differentiates into bulbous, invasive hyphae, which fill epidermal cells, invaginating the host plasma membrane and forming the specialised extra-invasive hyphal membrane (EIHM). Invasive hyphae also elicit formation of a specialised body, termed the Biotrophic Interfacial Complex (BIC), a plant membrane-rich structure. We are investigating the mode of secretion of effectors that function in the apoplastic space between the invasive hyphae and the EIHM and those that function within host cells. Apoplastic effectors include the Slp1 effector, which sequesters chitin oligomers released from the fungal cell wall that can otherwise be perceived by the CEBiP pattern recognition receptor. Cytoplasmic effectors include Avr-Pi-ta, Avr-Pia, Avr-Pii and Pwl2, which act as inducers of effector-triggered immunity in the presence of cognate resistance gene products. Preliminary evidence carried out using a combination of pharmacological, cell biological and genetic analysis, suggests that there may be different modes of exocytosis employed by *M. oryzae* to secrete effectors destined for delivery to these different host compartments. This work has been carried out in collaboration between the Talbot and Valent groups and complementary studies will be presented.

Translocation of oomycete RxLR-proteins

S2.4

PIETER VAN WEST, IRENE DE BRUIJN, SEVERINE GROUFFAUD, LARS LÖBACH, KIRSTY MINOR, CHRIS J. SECOMBES, STEPHAN WAWRA

21:10–21:45

p.vanwest@abdn.ac.uk

Aberdeen Oomycete Laboratory, University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK

The fish pathogenic oomycete *Saprolegnia parasitica* causes the disease Saprolegniosis and is responsible for devastating infections of salmonid fish in the aquaculture industry. Interestingly, *S. parasitica* has genes encoding secreted proteins with RxLR-like sequences. RxLR-proteins were first discovered in plant pathogenic oomycetes and were found to be able to translocate into host and non-host cells.
Therefore, we decided to investigate if and how the RxLR-like proteins from *Saprolegnia* are translocated into fish cells. In addition, we compared the findings based on the putative RxLR-like effectors from *S. parasitica* to results obtained from studies evaluating how plant pathogenic oomycetes translocate their RxLR-effector proteins.
We found that two RxLR-like proteins from *Saprolegnia* can enter fish cells in the absence of the pathogen and that the proteins localized into cytosolic vesicles. The translocation mechanism of the putative effectors from *Saprolegnia* appears to be host cell specific and independent of phospholipids. Instead, we found that the cell entry process is dependent on tyrosine-O-sulphation of their host cell surface receptor(s). Here our latest findings will be presented and discussed.

Session 3: Induction and suppression of host immunity by microbes
Chair: Sophien Kamoun

Modulation of plant immunity by oomycete effectors **S3.1**

SOPHIEN KAMOUN **08:05–08:50**

sophien.kamoun@tsl.ac.uk

The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

The major classes of molecular players both from plants (surface and intracellular immunoreceptors) and microbes (PAMPs and effectors) have now been revealed from several pathosystems, including plant pathogenic oomycetes such as the Irish potato famine organism *Phytophthora infestans*. These pathogens secrete a diverse repertoire of effector proteins that modulate host innate immunity and enable parasitic infection. Some effectors are targeted to the apoplast (apoplastic effectors), while others, notably the RXLR and CRN families, are translocated inside the host cell (cytoplasmic effectors). A number of RXLR effectors activate immunity in plants that carry cognate immunoreceptors of the NB-LRR class. Other oomycete molecules, such as elicitins, have features of PAMPs; they activate immunity via surface receptors and their modulator the receptor-like kinase BAK1/SERK3. We study several aspects of oomycete-plant interactions but this presentation will highlight the progress made by my lab and our collaborators in understanding how oomycete effectors modulate host immunity. Notably, we discovered that several effectors engage in complexes with host immune modulators to target specific pathways and suppress immunity. Remarkably, several RXLR effectors accumulate around haustoria to interfere with the execution of polarized host defenses. Our findings indicate that RXLR effectors form a unique toolkit to dissect focal responses at pathogen penetration sites.

Xanthomonas AvrBsT targets a microtubule-associated protein involved in immunity

S3.2

MARY BETH MUDGETT, MI SUN CHEONG, JUNG-GUN KIM, KEN FRAME, ANGELA KIRIK

08:50–09:25

mudgett@stanford.edu

Department of Biology, Stanford University, Stanford, CA, 94305, USA

The goal of this work is to elucidate the biochemical function of the *Xanthomonas* effector protein AvrBsT, which is translocated by the T3S system into plant cells during bacterial pathogenesis. AvrBsT is a putative acetyltransferase belonging to the YopJ-like effector family that is widely conserved in plant and animal bacterial pathogens. In previous work, we utilized the *Pseudomonas*-Arabidopsis model system to study AvrBsT-dependent immune signaling. AvrBsT action within Pi-0 plants results in the production of a phosphatidic acid burst that results in defense activation. Genetic studies revealed that resistance is due to a loss-of-function mutation in a conserved lipase designated as *SOBER1* – suppressor of AvrBsT-elicited resistance 1 (Cunnac et al. 2007). To understand the biochemical crosstalk between AvrBsT and SOBER1, we performed an interaction screen to identify Arabidopsis proteins that bind to both proteins. This work led to the identification of an unknown family of proteins that differentially bind to AvrBsT and SOBER1. One isoform is a microtubule-associating protein (MAP) required for PAMP-triggered immunity and effector-triggered immunity. AvrBsT perturbation *in planta* results in dynamic changes in the MAP localization during infection. Recent progress in understanding the biochemical and physiological consequences of the AvrBsT-MAP interaction will be presented.

Harnessing TAL effector-DNA targeting to understand and prevent plant diseases caused by *Xanthomonas*

S3.3

ADAM J. BOGDANOVE, R. ANDRES CERNADAS, ERIN L. DOYLE, AARON W. HUMMEL, CLARICE L. SCHMIDT, LI WANG

09:45–10:20

ajb7@cornell.edu

Department of Pant Pathology and Microbiology, Iowa State University, Ames, IA, USA; Department of Plant Pathology and Plant Microbe Biology, Cornell University, Ithaca, NY, USA

Transcription activator-like (TAL) effectors are type III-secreted, DNA binding proteins used by *Xanthomonas* to activate plant genes that promote infection. Some TAL effectors activate genes that confer resistance associated with host cell death. The proteins contain polymorphic repeats that assemble into a superhelix to track the DNA major groove and make base specific contacts. A TAL effector-DNA binding code that links individual repeat types to individual bases enables prediction or synthesis of TAL effector binding sites and customization of TAL effectors for binding new DNA sequences. Using the code and transcript profiling data, we identified multiple candidate targets in rice for TAL effectors of *X. oryzae* pv. oryzicola, which causes bacterial leaf streak of rice, and experimentally validated roughly half. Using TAL effector-based technologies, we discovered among these the first known gene for bacterial leaf streak susceptibility. And, we engineered a bacterial blight resistance gene to be activated by multiple TAL effectors from *X. oryzae* pv. oryzicola and *X. oryzae* pv. oryzae. As a stable transgene, this construct provided resistance against diverse strains of both pathovars. We also characterized the roles of several TAL effectors across rice genotypes using an otherwise TAL effector free strain to deliver them individually.

Multiple targeting of receptor kinase-mediated immunity by bacterial effectors

S3.4

CYRIL ZIPFEL

10:50–11:25

cyril.zipfel@sainsbury-laboratory.ac.uk

The Sainsbury Laboratory, Norwich Research Park, Norwich, UK

The first layer of plant innate immunity relies on the recognition of microbes via the perception of pathogen-associated molecular patterns (PAMPs) by surface localized pattern recognition receptors (PRRs) leading to PAMP-triggered immunity (PTI). In the plant model *Arabidopsis thaliana*, the leucine-rich repeat RKs (LRR-RKs) FLS2 and EFR are the PRRs for bacterial flagellin (or flg22) and elongation factor Tu (or elf18), respectively. Within seconds of PAMP binding, FLS2 and EFR form a ligand-induced complex with the regulatory LRR-RK SERK3/BAK1 leading to phosphorylation of both proteins. Additional SERKs, such as SERK4/BKK1, are recruited in a ligand-dependent manner into EFR and FLS2 protein complexes with different preferences. FLS2 (and potentially EFR) also forms a constitutive complex with the membrane-associated cytoplasmic kinase BIK1 that get phosphorylated in a BAK1-dependent manner upon PAMP binding. BIK1 is a positive regulator of most FLS2- and EFR-mediated responses. Pathogens must block or avoid PTI to cause disease. A potent strategy to inhibit PTI is via the action of secreted effectors delivered into the host cells leading to effector-triggered susceptibility (ETS). The genome of the phytopathogenic model bacterium *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000) encodes >30 type III-secreted effectors (T3SEs). Recently, several T3SEs from different *P. syringae* strains were shown to be virulence factors. Corresponding host targets have been identified only for a few of them, but they revealed that T3SEs interfere with key components of PTI. Here, we will present two novel strategies used by *Pto* T3SEs to inhibit FLS2- and EFR-mediated immunity, illustrating that pathogens evolved multi-layered approaches to successfully impede immunity.

Effector-induced modification of plant immune signaling **S3.5**

GITTA COAKER **11:25–12:00**

glcoaker@ucdavis.edu

Department of Plant Pathology, University of California- Davis, Davis, CA, USA

The plant innate immune system is capable of recognizing diverse microbial patterns and pathogen effectors through intracellular and surface-localized immune receptors. Plant pathogenic bacteria possess arsenals of 20-40 effectors that are delivered into host cells during infection. The role of two *Pseudomonas syringe* effectors, AvrB and HopQ1, will be presented. HopQ1 is a conserved effector than can suppress plant immunity in both Arabidopsis and tomato, is phosphorylated *in planta,* and interacts with host 14-3-3 proteins. The AvrB effector is recognized by the RPM1 NLR immune receptor and induces RIN4 phosphorylation. The role of RIN4 phosphorylation in triggering activation of the *Arabidopsis* immune receptor RPM1 will be presented. The importance of RIN4 phosphorylation during compatible interactions will also be presented. Posttranslational modification of both pathogen effectors and plant proteins play critical roles in early immune signaling.

Probing jasmonate receptor signaling using small-molecule effector coronatine

S3.6

SHENG YANG HE

12:00–12:35

hes@msu.edu

Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA; Howard Hughes Medical Institute; Gordon-Betty Moore Foundation; USA

We have been studying how *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000 causes disease in *Arabidopsis thaliana*. During infection, *Pst* DC3000 produces a battery of virulence factors to engage multiple host cell types and diverse host physical and chemical barriers. The bacterial type III secretion system (T3SS) delivers 'effector' proteins directly into the host cell, whereas the phytotoxin coronatine mimics the active form of plant hormone jasmonate. Study of the molecular action of T3SS effectors and coronatine has begun to show the great utility of bacterial pathogenesis as a probe in the discovery of new components of the plant immune system, as well as fundamental cellular mechanisms in plants. In this talk, I will discuss our research that contributed to the identification of the jasmonate receptor complex and the understanding of the molecular mechanisms by which coronatine suppresses host defenses and how plants coordinate growth-defense tradeoffs during jasmonate signaling activation. I will also attempt to discuss the need and associated challenges for innovative approaches to inactivate the molecular action of coronatine and T3SS effectors.

Session 4: Structural biology of microbial effectors and their targets
Chair: Jeff Dangl

Plant immune system function and its battle with pathogen effectors

S4.1

JEFF DANGL

19:30–20:15

dangl@email.unc.edu

HHMI/Dept. of Biology and Carolina Center for Genome Sciences, University of North Carolina at Chapel Hill, NC27599, USA

The plant immune system is a sophisticated two-tiered receptor-based system that utilizes transmembrane pattern recognition receptors to survey microbe associated molecular patterns (MAMPs). Recognition mediated by this 'first tier' of the plant immune system generates a set of downstream responses that are sufficient to stop the growth of most microbes, termed MTI (MAMP-triggered immunity). Pathogens, by definition, can surpass or evade MTI and they do so by deploying into the host cell suites of virulence effectors. Plants have therefore evolved a second, intracellular receptor system, consisting of NLR proteins, which monitor effectors directly as ligands, or via their alteration of NLR-associated host targets. Activation of an NLR essentially 're-boots' suppressed MTI, but does so in a very rapid and high amplitude manner termed ETI (Effector-triggered immunity). One first set of goals are to understand how particular pathogen effector proteins have evolved to manipulate host signaling machinery to function as virulence factors; how these molecular manipulations are recognized by the intracellular NLR receptors; and how NLR activation initiates a successful immune response. Our rationale is that by understanding how a broad collection of virulence factors from evolutionarily diverse pathogens act inside the host cell, we will better understand the normal defense relevant function of their targets.

Protein structures at the interface between type III effectors and the plant immune system

S4.2

GREGORY MARTIN[1], JIJIE CHAI[2], PATRICK BOYLE[1], JOHANNES MATHIEU[1], KATHY MUNKVOLD[1], HAISHAN GAO[2], SHA WANG[2], YONG-BIN YAN[2], JINJING WANG[2], WEI CHENG[2], HANH NGUYEN[1], INHWA YEAM[1], SIMON SCHWIZER[1]

20:15–20:50

gbm7@cornell.edu

[1]Boyce Thompson Institute for Plant Research and Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, New York, USA; [2]Key Laboratory for Protein Sciences of Ministry of Education, School of Biological Sciences, Tsinghua University, Beijing, China

Pseudomonas syringae pv. *tomato* (*Pst*) causes bacterial speck disease of tomato by utilizing its type III secretion system to deliver ~30 virulence-promoting proteins ('effectors') into the plant cell. Two of these effectors, AvrPto and AvrPtoB, act early in the infection process by interfering with the kinase domains of MAMP receptor complex proteins (e.g. FLS2/BAK1). This interference in defense signaling appears to then enable the virulence activities of later-acting effectors. Some tomato varieties are immune to speck disease because they express the Pto kinase that interacts with either AvrPto or AvrPtoB and acts in concert with the NBARC-LRR protein Prf to activate a strong defense response. Fen, another kinase related to Pto, recognizes forms of AvrPtoB that are missing the C-terminal domain. Over the past 10 years, protein structural biology relying on NMR and crystallography and functional analyses have revealed the underlying basis for how host immunity-associated kinases (BAK1, Fen, and Pto) interact with various domains of AvrPto and AvrPtoB. These insights have led to the development of a model for the evolutionary processes that have shaped the tomato-*Pst* interaction. Current work is focused on integrating knowledge of the structures of effectors and host kinases with what we are learning about natural variation in *Pst* recognition from characterization of wild tomato species. Supported by NIH R01GM078021, NSF-0841807, NSF-1025642, and USDA-2010-65108-20503.

Structural insights into TIR domain and effector function in effector-triggered immunity in flax and Arabidopsis

S4.3

BOSTJAN KOBE[1], THOMAS VE[1], SIMON WILLIAMS[1,2], LI WAN[1], MAUD BERNOUX[3], PRADEEP SORNARAJ[2], EMMA DE COURCY-IRELAND[2], JEFFREY G. ELLIS[3], PETER A. ANDERSON[2], PETER N. DODDS[3]

b.kobe@uq.edu.au

[1]School of Chemistry and Molecular Biosciences, Institute for Molecular Bioscience, and Centre for Infectious Disease Research, University of Queensland, Brisbane 4072, Australia; [2]School of Biological Sciences, Flinders University, G.P.O. Box 2100, Adelaide 5001, Australia; [3]CSIRO Plant Industry, Canberra, Australia

21:05–21:40

Effector-triggered plant immunity is initiated through the recognition of a pathogen effector protein by a plant resistance (R) protein, leading to the activation of plant defenses and a localized cell death response. The effectors usually have roles in virulence and are structurally diverse, while R proteins generally fall into a few conserved families. We have used the fungal pathogen flax rust interaction with flax as a model system to characterize this process. We have shown a direct interaction of the effector proteins AvrL567 and AvrM with R proteins L6 and M, respectively, and also determined the crystal structures of AvrL567 and recently AvrM. The structure of AvrM reveals surface-exposed hydrophobic residues that may mediate uptake of this effector into the plant cell, as well as residues that may mediate interaction with the R protein M. We further determined the crystal structure of a TIR domain from L6. The structure highlights three separate functionally important protein surfaces, involved in oligomerization, interaction with a downstream signaling partner, and regulatory intramolecular interactions, respectively. We have complemented this work with a study of the TIR domains of Arabidopsis R proteins RPS4 and RRS1, which work in concert to confer resistance to several pathogens. We show that these TIR domains interact directly in vitro, and determined the crystal structures of these TIR domains individually and in the heterodimer complex. Our results bring us a step closer to understanding the molecular basis for the disease resistance process.

Structure-led studies of plant pathogenic effector proteins **S4.4**

MARK J. BANFIELD **21:40–22:15**

mark.banfield@jic.ac.uk

Dept. of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK

In the absence of significant sequence conservation, structural biology offers unique opportunities to discover functional and evolutionary relationships in proteins. Some of the world's most devastating plant pathogens use secreted effector proteins to suppress host defence mechanisms and manipulate other cellular processes for the benefit of the pathogen. These effector proteins can be secreted to the plant apoplast or translocated into host cells. From these locations, effector proteins can also by recognized by hosts and this can result in an immune response that limits pathogen growth and dissemination. Using both model and crop-relevant pathosystems, my Lab investigates the three dimensional structure and function of effector proteins. This includes bacterial effectors delivered by the pathogen-encoded type-3 secretion system and oomycete RXLR effectors. We use structural biology to inform functional annotation of effector virulence activities, define evolutionary relationships and provide insights into how effectors can trigger host immune responses. We also employ biochemical and *in planta* studies to understand effector function, including macromolecular interactions and subcellular localization. In my talk I will discuss some specific recent advances in these areas made in my Laboratory.

Session 5: Emerging systems
Chair: Francis Martin

The mutualistic fungus *L. bicolor* uses the effector protein MiSSP7 to alter host jasmonate signaling

S5.1

J.M. PLETT[1], Y. DAGUERRE[1], A. DEVEAU[1], A. KOHLER[1], J. MORRELL-FALVEY[2], A. BRUN[1], F. MARTIN[1]

08:05–08:50

fmartin@nancy.inra.fr

[1]Lab of Excellence ARBRE, INRA-Lorraine University, UMR 'Interactions Arbre/micro-organismes', INRA-Nancy, 54280 Champenoux, France; [2]Oak Ridge National Laboratory, Oak Ridge, TN 37831-6422, USA

Ectomycorrhizal fungi have helped shape forest communities worldwide over the last 180 million years through a mutualistic relationship with tree roots in which the fungal partner provides a large array of nutrients to the plant host in return for photosynthetically derived sugars. Like pathogenic organisms, the mutualistic basidiomycete *Laccaria bicolor* appears to utilize effector proteins to aid the colonization of plant tissues, although the plant targets of a large majority of these effectors have yet to be identified. MiSSP7, the most highly symbiosis-upregulated gene from *L. bicolor* encodes an effector protein indispensible for the establishment of mycorrhizal mutualism. MiSSP7 is secreted by the fungus upon receipt of diffusible signals from plant roots, imported into the plant cell via endocytosis, and targeted to the plant nucleus where it alters the transcriptome of the plant cell. Here we show that MiSSP7 interacts with the jasmonic acid receptor JAZ6 of *Populus trichocarpa*. Further, we demonstrate that PtJAZ6 interacts with a number of other nuclear based proteins to form a DNA binding complex. We show that MiSSP7 is able to block jasmonic acid signaling in both *L. bicolor* host and non-host plants, likely through its interaction with JAZ receptors. *L. bicolor* transformants with severely reduced expression of MiSSP7 do not enter into symbiosis with poplar roots, a phenotype that can be complemented by transgenically varying the transcription of PtJAZ6 or through inhibiting jasmonic acid biosynthesis. We conclude, based on our results, that MiSSP7 is an effector protein used to promote mutualism by blocking jasmonic acid signaling through the PtJAZ6 receptor during plant colonization. Given the key results obtained for MiSSP7, the role played by other ectomycorrhiza-upregulated small secreted proteins of *L. bicolor* and other sequenced mycorrhizal fungi should be elucidated, as well as the identity of plant-based signals that may control *L. bicolor* growth within the root space.

Plant cell reprogramming during arbuscular mycorrhizal symbiosis

S5.2

NATALIA REQUENA **08:50–09:25**

natalia.requena@kit.edu

Molecular Phytopathology Department, Karlsruhe Institute of Technology, Hertzstrasse 16, D-76187 Karlsruhe, Germany

Plant roots are constantly approached by a myriad of microorganisms and are thus challenged to recognize friends from foes. Most plant roots engage in a mutualistic association with fungi from the Glomeromycota Phylum forming the arbuscular mycorrhiza (AM) symbiosis. The establishment of this beneficial association requires an intensive signal exchange including the down-regulation of PAMP triggered responses. We have shown that secretion and delivery of the effector protein SP7 contributes to the manipulation of the plant defense response. However, we have additional evidence that shows that this might be only one of the mechanisms used by AM fungi and that several other signals contribute to the full modulation of the plant cell program.

Functional characterization of effector proteins that modulate plant–insect interactions

S5.3

SASKIA HOGENHOUT

09:45–10:20

saskia.hogenhout@jic.ac.uk

Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK

Sap-feeding aphids and leafhoppers cause feeding damage and transmit a number of plant diseases, including viruses and bacterial pathogens such as phytoplasmas. We found that plant basal defence pathways are induced upon aphid attack and that specific aphid effectors suppress these plant defences. Furthermore, silencing of a number of candidate effector genes in aphids by plant-mediated RNAi reduces aphid performance, while expression of these effectors in plants increases aphid progeny production. Aphid effectors are under positive selection to promote aphid colonization on specific plant species.

The obligate leafhopper-transmitted phytoplasmas have effectors that promote leafhopper colonization thereby increasing the chance of phytoplasma transmission to other plants. Phytoplasma effector protein SAP11 destabilizes TCP plant transcription factors resulting in increased stem production and altered leaf development and reduced jasmonate (JA) synthesis, while effector protein SAP54 destabilizes MADS-box transcription factors leading to the conversion of flowers into leaves and delayed plant senescence. Leafhoppers feed and lay eggs on green plant tissues and are sensitive to JA. Both phytoplasma effectors promote leafhopper feeding and reproduction contributing to a 60% increase in the number of insect vectors on phytoplasma-infected plants.

Thus, both insects and insect-transmitted pathogens produce effectors that promote insect colonization on plants.

Cyst nematode effectors: localization, host targets, and functions in plant parasitism

S5.4

MELISSA G. MITCHUM

goellnerm@missouri.edu

Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA

10:50–11:25

Nematode stylet-secreted effectors (SSEs) produced in the dorsal and subventral oesophageal gland cells are delivered into host tissues to promote parasitism. Recent advances in microgenomics and proteomics have removed previous research barriers to acquire sufficient quantities of gland contents and stylet secretions suitable for molecular and biochemical analyses. Consequently, a large number of phytonematode effectors have been identified and characterized. Among these, small peptides with potential regulatory roles that influence plant development have been discovered. A majority of the effectors identified are novel proteins, but recent studies directed at identifying the host targets of these effector proteins have provided new insight into their potential functions. In this talk, I will summarize current knowledge about the localization, host targets, and putative functions of nematode peptides and several novel effectors in plant parasitism.

Investigating virulence effectors in the poplar-poplar rust pathosystem

S5.5

SÉBASTIEN DUPLESSIS[1], BENJAMIN PETRE[1], HUGO GERMAIN[2,3], ARNAUD HECKER[1], PASCAL FREY[1], FABIEN HALKETT[1], STÉPHANE DE MITA[1], DAVID L JOLY[2,4], STÉPHANE HACQUARD[1], ARMAND SÉGUIN[3], NICOLAS ROUHIER[1]

11:25–12:00

duplessi@nancy.inra.fr

[1]UMR 1136 Interactions Arbres/Microorganismes, INRA/Université de Lorraine, Centre INRA de Nancy, Champenoux, France; [2]Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Québec, QC, Canada; [3]Université du Québec à Trois-Rivières, Trois-Rivières, QC, Canada; [4]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, Canada, Canadian Forest Service, Laurentian Forestry Centre, Québec, Canada

Foliar rust caused by *Melampsora larici-populina* is a major disease affecting poplar plantations throughout the world. The obligate biotrophic status of the fungus and the perennial status of the host plant, make molecular investigation of this interaction a real challenge. However, availability of both *Populus trichocarpa* and *Melampsora larici-populina* sequenced genomes has allowed for setting an emerging model pathosystem to decipher the molecular bases of disease resistance in trees and of biotrophic growth in rust fungi. Comprehensive analyses of rust transcripts encoding small secreted proteins expressed *in planta* identified putative virulence factors and we focused our attention on a few candidates that show evidence of purifying selection between paralogs. Currently, we combine a range of approaches including biochemical and structural characterization of recombinant proteins, virulence assays in an heterologous Arabidopsis-*Pseudomonas syringae* pathosystem, yeast two-hybrid and pull-down assays to characterize these candidate effectors. We use population genomics as well to narrow the identification of new virulence candidates. Genome sequencing of poplar rust isolates selected in collections sampled over ten years in natural and cultivated poplar stands all over France around major events of resistance breakdown in plantations is used for this purpose. I will present a broad overview of our current knowledge of the pathosystem and progress we made to identify and characterize candidate virulence factors.

How oomycete pathogens of Arabidopsis cause or fail to cause disease

S5.6

JONATHAN D. G. JONES, ERIC KEMEN, ARIANE KEMEN, KEE-HOON SOHN, GEORGINA FABRO, OLIVER FURZER, ALEX ROBERT-SEILANIANTZ, NAVEED ISHAQUE, JORGE BADEL, MARIE-CECILE CAILLAUD, LENNART WIRTMUELLER, MARK BANFIELD, SOPHIE PIQUEREZ

12:00–12:35

jonathan.jones@sainsbury-laboratory.ac.uk

Sainsbury Lab, Norwich, UK

Plant disease resistance mechanisms are initiated by surface receptors and cytoplasmic receptors that respectively recognize conserved or variable pathogen components. To suppress defence, pathogens deliver effector molecules into host cells. Understanding these effectors is important to identify new probes to host defence mechanisms and develop durable resistance strategies. Although the effector complements of bacteria are becoming well defined, and the mechanisms of many bacterial effectors are quite well understood, the effectors of the fungal and oomycete pathogens that cause the most serious crop losses are still poorly characterized.
As a model system, we work with the downy mildew pathogen *Hyaloperonospora arabidopsidis (Hpa)* and two other oomycete pathogens, *Albugo laibachii and A candida*. The *Hpa* genome is available. We used Illumina paired read sequencing to assemble sequences of multiple races of *Albugo laibachii*, a pathogen that is particularly effective at shutting down host defence, and also of multiple *A candida* races. We are using association genomics to correlate genetic variation in the secretome of *Albugo laibachii* with virulence or avirulence on specific Arabidopsis accessions. In addition, we are using the MAGIC inbred lines of Kover and Mott, to reveal transgressive segregation for susceptibility to *Brassica*-infecting *A. candida* strains, in order to identify genes for non-host resistance. An update on recent progress will be presented.

Kemen E, et al Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of *Arabidopsis thaliana* (2011) **PLOS Biology** 9 (7) pp. e1001094
Baxter L, Tripathy S, Ishaque N, et al Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome. (2010) **Science** 330: 1549−1551.

Hunting for the witch's effectors: Genomic and transcriptomic analyses of the parasitic *Orobanchaceae* plants

S5.7

SATOKO YOSHIDA, JULIANE ISHIDA, TAKANORI WAKATAKE, RIICHIRO MANABE, KEN SHIRASU

12:35–13:10

ken.shirasu@psc.riken.jp

RIKEN, Plant Science Center, Japan

Parasitic plants belonging to the family *Orobanchaceae* have emerged as serious threats in agriculture. For example, *Striga hermonthica*, the witchweed, is an obligate root parasite that infects economically important crops such as sorghum, maize, millet, and upland rice in sub-Saharan Africa, and the yield losses caused by this species have been estimated to cost as much as US$ 7 billion annually. Despite its agricultural importance, molecular mechanisms controlling the establishment of parasitism are poorly understood. To understand of the parasitism, we initiated large-scale genome and transcriptome analyses of *S. hermonthica* and its close relative *S. asiatica*. We have also developed a model system to understand the parasitism using the hemiparasite *Phtheirospermum japonicum* belonging to *Orobanchaceae. P. japonicum* can be easily grown in the lab and is amenable for various genetic analyses, such as crossing, mapping and transformation. The transcriptome analysis has provided a list of genes that are specifically expressed during infection and encode secreted proteins, as effector candidates.

Poster Abstracts

Poster abstracts are listed alphabetically by first author, presenting author is underlined.

P1 A plant-derived signal induces expression of the type III secretion system in *Pseudomonas syringae* and is genetically regulated by Arabidopsis *Map Kinase Phosphatase 1*

JEFFREY C. ANDERSON, YING WAN, SCOTT C. PECK

Department of Biochemistry, University of Missouri, Bond Life Science Center, 1201 Rollins St., Columbia, MO 65211, USA

Map Kinase Phosphatase 1 (*MKP1*) is a negative regulator of resistance against *Pseudomonas syringae* pv tomato (*Pto*) DC3000 in Arabidopsis. Although multiple defense-associated responses are enhanced in a loss-of-function *mkp1* mutant, the mechanism(s) of heightened resistance is unknown. To investigate the underlying cause of this resistance, we measured the delivery of a type III (T3) effector protein into *mkp1* cells by *Pto* DC3000 using an adenylate cyclase reporter. Interestingly, T3 effector delivery was significantly reduced in *mkp1*, suggesting that weak effector delivery may contribute to the decreased virulence of *Pto* DC3000 observed in *mkp1*. Based on this result, we hypothesize that enhanced *mkp1* resistance may be due to heightened plant defenses that block T3 delivery, or weaker deployment of the T3 secretion system (T3SS) in *Pto* DC3000. In support of the latter hypothesis, we recently discovered the presence of a chemical signal in tomato and Arabidopsis plants that strongly induces T3SS gene expression in *Pto* DC3000. Surprisingly, *mkp1* plants have reduced levels of this T3SS-inducing signal, suggesting that loss of a virulence-promoting signal may contribute to the decreased virulence of *Pto* DC3000. Our progress towards identifying and characterizing the T3SS-inducing signal and its role in *mkp1* resistance will be presented.

P2 Potato aphid salivary secretome: Pandora's box

HAGOP S. ATAMIAN[1], RITU CHAUDHARY[1], VALERIANO DAL CIN[1], ZHOUXIN SHEN[2], STEVEN P. BRIGGS[2], ISGOUHI KALOSHIAN[1]

[1]Graduate Program in Genetics, Genomics and Bioinformatics, Department of Nematology, University of California, 900 University Ave, Riverside, CA 92521, USA; [2]Division of Biological Sciences, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093, USA

The interaction between aphids and their host plants seems to be analogous to those of plant–microbial interaction. Unlike microbial effectors, little is known about aphid effectors and their ability to interfere with host immunity. To identify potato aphid (*Macrosiphum euphorbiae*) effectors, we used two distinct approaches: developed a salivary gland transcriptome using Illumina technology and subjected aphid saliva to mass spectrometry. We generated 85 million paired-end Illumina reads from salivary glands and assembled them into 646 contigs. *Ab initio* sequence analysis predicted secretion signal peptide in 24% of these sequences suggesting they might be secreted into the plant during aphid feeding. 31% of the salivary gland sequences with secretion signal peptide were present in the aphid saliva. Interestingly, among the salivary proteome were proteins originating from the aphid endosymbiont *Buchnera aphidicola* suggesting movement of proteins from the insect hemocoel into the salivary glands and questioning the evaluation of aphid secreted proteins simply based on the presence of secretion signal. Several candidate effectors with secretion signal peptide were functionally characterized using *Agrobacterium tumefaciens*-mediated transient overexpression in *Nicotiana benthamiana* or delivering them into tomato through *Pseudomonas syringae* type three-secretion system. Aphid effectors with the ability to manipulate host defenses were identified.

P3 Functional characterization of *Pseudomonas syringae* type III effectors in Arabidopsis

K. AUNG, S. Y. HE

Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA; Howard Hughes Medical Institute-Gordon and Betty Moore Foundation, Chevy Chase, MD 20815, USA

Pseudomonas syringae pv. *tomato* (*Pst*) DC3000 injects ca. 30 effector proteins into host plant cells through the type III secretion system upon infection. As determining the subcellular localization of these effectors in host cells and identification of their host targets are essential steps toward understanding their function, we are systematically examining the subcellular localization of *Pst* DC3000 effectors. To this end, yellow fluorescent protein (YFP) was tagged to the N- or C-terminal end of 17 effectors that have not received extensive studies and the fusion proteins were transiently expressed in *Nicotiana tabacum*. Most YFP-effectors were detected in the cytoplasm and the nucleus; however, some effectors are observed on the plasma membrane, mitochondria, or the endoplasmic reticulum. As a pilot experiment, we have stably expressed HopO1-1, a putative ADP-ribosyltransferase, in Arabidopsis. Our results showed that YFPHopO1-1 is detected in both the cytoplasm and the nucleus, whereas HopO1-1-YFP was observed mainly on the plasma membrane. To identify the host target proteins of HopO1-1, the transgenic plant expressing a functional fusion protein will be subjected to pull-down assays using GFP-Trap. If our pilot experiment is proven to be a valid and efficient method, the same approach will be adopted to analyse the molecular function of the other 16 effectors.

P4 Molecular and genomic strategies to engineer durable and sustainable disease resistance to cassava bacterial blight

REBECCA BART[1], TITUS ALICAI[2], ADRIANA BERNAL[3], MEGAN CASEY[1], DOUG DALBECK[1], ANDREW KASSEN[1], LAVA KUMAR[4], CESAR MEDINA[3], LEANDRO MOREIRA[5], JÚLIO RODRIGUES NETO[6], ANNALISE PETRIELLO[1], MIKEL SHYBUT[1], BRIAN STASKAWICZ[1]

[1]University of California, Berkeley, USA; [2]The National Crops Resources Research Institute (NaCRRI), Namulonge, Kampala, Uganda; [3]Universidad de los Andes, Bogotá, Colombia; [4]International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan, Nigeria; [5]Universidade Federal de Ouro Preto, Brazil; [6]IBSBF, Brazil

The bacterial blight disease of cassava (CBB) incited by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) is a serious threat to cassava production in several developing countries in both Africa, Asia and South America. Currently, bacterial blight of cassava is extremely difficult to control, as it has been very problematic to breed for resistance. Our primary objective is to develop durable and effective resistance to CBB. To accomplish this goal, we have undertaken a population scale genome characterization study of *Xam* to sequence, annotate, and characterize the constellation of bacterial Type Three Secretion System (TTSS) effector proteins among naturally occurring field isolates of this important bacterial pathogen. We have generated genome sequences for 65 geographically and temporally diverse *Xam* strains using Illumina technology. With a novel effector prediction pipeline, we have identified the most conserved *Xam* effectors which will be useful in identifying novel sources of resistant cassava germplasm. In addition, we are developing a new system of engineering resistance as follows: the *Xam* genome encodes members of the TAL (Transcription Activator-Like) family of effectors. These effector proteins are delivered to the plant nucleus via the TTSS and act as eukaryotic transcriptional activators by binding to specific nucleotide recognition sequences termed Effector Binding Elements (EBEs). We have cloned the EBE sequence upstream of effector genes that trigger non-host resistance in cassava such that, during *Xam* pathogenesis, the TAL effectors trigger a resistance response. We are working to make transgenic plants containing these constructs. The development of novel and durable resistant varieties that recognize the most conserved TALEs will have a broad impact on farmers in developing countries as the novel transgenes that we propose to construct can be introduced into the many different varieties that are currently being planted. Thus, the development of CBB-resistant varieties will have enormous implications for food security in countries in which cassava is a major staple food.

P5 Amino acid metabolism in plant pathogen resistance

FRIEDERIKE E.M. BERNSDORFF[1], HANA NAVÁROVÁ[1,2], JÜRGEN ZEIER[1,2]

[1]Department of Biology, Heinrich Heine University, Universitätsstraße 1, D- 40225 Düsseldorf, Germany;
[2]Plant Biology Section, University of Fribourg, Route Albert Gockel 3, CH-1700 Fribourg, Switzerland

After pathogen infection, a massive reprogramming of the plant takes place at the transcriptional and metabolic levels. For instance, *Pseudomonas syringae* pv. *maculicola (Psm)* infection alters the concentration of amino acids in inoculated and in distal, non-inoculated Col-0 leaves. Levels of aliphatic and aromatic amino acids significantly increase upon pathogen infection. Moreover, the levels of lysine and, in particular, levels of the lysine catabolites α-aminoadipidic acid (Aad) and pipecolic acid (Pip) substantially increase in inoculated leaves. Pip does not only accumulate at inoculation sites but also in leaves distant from initial pathogen contact. Previous experiments by Navárová and Zeier indicate that the lysine aminotransferase ALD1 mediates pathogen-induced Pip biosynthesis and demonstrate that Pip is a critical regulator of several forms of inducible plant immunity, including basal, specific and systemic acquired resistance (SAR). Apart from ALD1, it is not known which genes and enzymes are involved in the production of Pip and possible intermediates and conversion products are not well-defined. Microarray analyses reveal that a number of amino acid-related genes encoding predicted aminotransferases and reductases are up-regulated after *Psm* treatment. Metabolite analyses and pathophysiological experiments with selected knockout lines are now performed to further explore pathogen-induced amino acid metabolism. Furthermore, we are investigating the functional relationship between the two essential SAR-regulatory metabolites salicylic acid (SA) and Pip.

P6 Identification of *Medicago truncatula* symbiotic genes preventing plant defense reactions during symbiosis

MARIE BOURCY[1], LYSIANE BROCARD[1,3], CATALINA PISLARIU[2], VIVIANE COSSON[1], PETER MERGAERT[1], KIRANKUMAR MYSORE[2], MICHAEL UDVARDI[2], BENJAMIN GOURION[1], PASCAL RATET[1]

[1]*Institut des Sciences du Végétal, CNRS, Avenue de la Terrasse, 91198 Gif-Sur-Yvette, France;* [2]*Samuel Roberts Noble Foundation, Ardmore, OK, USA;* [3]*Present address Lysiane BROCARD, Bordeaux Imaging Center, Pôle d'Imagerie du Végétal IBVM, INRA, 71 avenue Édouard Bourleaux, 33883 Villenave d'Ornon, France*

Medicago truncatula interacts with rhizobia to establish nitrogen fixing symbiosis. During the symbiotic relationship, the plants homes hundreds of bacteria into its symbiotic cells without triggering defense reactions. To understand this phenomenon, we selected a mutant line producing brownish nodules suggesting an alteration of symbiotic repression of plant defense reactions. The tagged gene responsible for this phenotype was identified using reverse genetics and crossing experiments. The mutant lines were not affected in organogenesis and no alteration of the symbiotic process was observed before bacteria were released into the symbiotic cells. However bacteria did not properly differentiate into the nitrogen fixing form and these symbiosomes rapidly became aberrant. Remarkably, living bacteria were detectable in only a few layers of symbiotic cells and this phenomenon correlated with the induction of plant defense reactions. By an *in silico* approach a second plant gene likely involved in the same process was identified. The requirement of this gene for nitrogen fixation was demonstrated using the corresponding mutant line. Interestingly, nodules of this mutant accumulate phenolic compounds as well. The identification of these two genes constitutes a significant step in the elucidation of the mechanism responsible for symbiotic repression of plant defense reactions.

P7 Impact of the PGPB *Enterobacter radicincitans* DSM16656 on growth, glucosinolate profile and immune responses of *Arabidopsis thaliana*

A. K. BROCK, B. BERGER, I. MEWIS, S. RUPPEL

Leibniz-Institute of Vegetable & Ornamental Crops Grossbeeren/Erfurt e.V.,Theodor-Echtermeyer-Weg 1, 14979 Grossbeeren, Germany

Growth and yield of various vegetable plants was shown to be significantly increased after the application of the plant growth promoting bacterial (PGPB) strain *Enterobacter radicincitans* DSM16656. Here, we focused our studies on the bacterial induced growth promotion in the non-crop plant *Arabidopsis thaliana* and analyzed the bacterial ability to affect the glucosinolate profile and plant defense responses. HPLC analysis revealed growth stage dependent altered contents of aliphatic glucosinolates in *E. radicincitans*-colonized plants. This effect seems to be correlated to developmental stage depending nitrogen requirement. Quantifying the transcription level of defense related genes *PR1* and *PDF1.2* using quantitative real-time PCR we found a first indication for *E. radicincitans* induced priming of defense responses in *A. thaliana*. Additionally, nitrogen deficiency studies suggest that plant nitrogen nutrition influences the intensity of plant growth enhancement by *E. radicincitans*.

P8 Genome sequencing and expression profiling of emerging strains of *P. infestans*

LILIANA M. CANO, SYLVAIN RAFFAELE, DAVID E. L. COOKE, RICARDO F. OLIVA, MARINA PAIS, GRAHAM ETHERINGTON, PAUL R. J. BIRCH, MICHAEL COFFEY, SOPHIEN KAMOUN

The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK; The James Hutton Institute JHI, Invergowrie, Dundee, DD2 5DA, UK

In 2005, a clonal lineage of the potato late blight pathogen *P. infestans*, genotype blue 13_A2, was identified in the UK. The increased aggressiveness of this genotype and its virulence on several resistant potato varieties led to it becoming the most prevalent genotype in UK within just a few years. In the US, genotype US22 caused a massive epidemic on tomato during the summers of 2009 and 2010 in Eastern USA and Canada.
To fully characterize the genetic basis for the success of these genotypes, we performed genome sequence and gene expression analysis. We discovered that the blue 13 strain, 06_3928A, exhibits significant genetic and gene expression polymorphisms. In addition, we found that 06_3928A exhibits a sustained gene induction pattern and an extended biotrophic growth phase during infection. Interestingly, 06_3928A carries intact and *in planta* induced *Avrblb1*, *Avrblb2* and *Avrvnt1* genes and is avirulent on potato lines that carry *Rpi-blb1*, *Rpi-blb2* and/or *Rpi-vnt1.1* genes. For the US22 strain, P17777, although this genotype is more aggressive on tomato when compared to the reference strain T30-4, our gene expression analysis showed that the majority of genes in P17777 were induced in both potato and tomato. This result indicates that the P17777 may be "host blind" and that without management US22 could also become a threat to potato. Our findings illustrate how pathogen genome analysis can assist with the management of destructive plant disease epidemics.

P9 Investigating the manipulation of ABA signalling by *Pseudomonas syringae* type III effectors

F. Y. CAO, S. LUMBA, S. TOH, M. TANIGUCHI, P. MCCOURT, K. YOSHIOKA, D. DESVEAUX

Department of Cell and Systems Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario, M5S 3B2, Canada

The phytopathogen *Pseudomonas syringae* delivers type III effectors (T3Es) into host cells to increase virulence by means of suppressing host defence. T3Es are capable of targeting hormone signalling pathways to perturb hormone homeostasis inside the host. Recent studies have elucidated the role of phytohormones including abscisic acid (ABA) in the interaction between various phytopathogens and their plant hosts. We aim to investigate mechanisms involved in the manipulation of ABA signaling by *P. syringae* T3Es. Physical interactions between *P. syringae* T3Es and Arabidopsis proteins that are transcriptionally regulated by ABA were identified using high throughput yeast-two-hybrid analyses. As expected, the interactome contains Arabidopsis proteins of high interconnectivity (hubs), many of which are targeted by T3Es. We will present our analysis of this plant-pathogen interactome as well as our efforts to identify T3Es that manipulate ABA signalling to promote *P. syringae* virulence.

P10 The *Magnaporthe oryzae* effectors Avr-Pia and AvrCO39 are recognized by the rice Nucleotide-Binding Site - Leucine Rich Repeat (NBS-LRR) protein RGA5 through direct interaction

S. CESARI[1], R. TERAUCHI[2], J. L. NOTTEGHEM[1], T. KROJ[1]

[1] Unité Mixte de Recherche, BGPI, Campus International de Baillarguet TA A54/K, 34398 Montpellier cedex 5, France; [2]Iwate Biotechnology Research Center, Kitakami, Iwate 024-0003, Japan

Plant immunity strongly relies on the recognition of pathogen effectors by plant resistance proteins. This recognition activates disease resistance signaling pathways leading to the inhibition of pathogen growth and the induction of a localized hypersensitive response. To better understand the molecular mechanisms governing effector recognition, we study two translocated effectors from the rice blast fungus *Magnaporthe oryzae*: Avr-Pia and AvrCO39. We show that both effectors are recognized by the products of the same duo of rice NB-LRR-coding genes, *RGA4* and *RGA5*. Interestingly, *RGA5* is subject to alternative splicing leading to the production of two protein isoforms termed RGA5-A and RGA5-B. Yeast two hybrid analysis revealed that Avr-Pia specifically interacts with a small RGA5-A specific domain whereas AvrCO39 interacts with both RGA5-A and RGA5-B. Genetic analysis indicates that RGA5-A recognizes, together with RGA4, AvrCO39 and Avr-Pia, while RGA5-B is inactive. This suggests that RGA5-A acts as receptor mediating recognition of the effectors by direct binding while RGA4 might act as a signaling component activating downstream resistance pathways.
Recent advance in the *in planta* validation of the observed interactions will be presented.

P11 An abietane diterpenoid is a potent inducer of systemic acquired resistance in plants

RATNESH CHATURVEDI[1], BARNEY J. VENABLES[1], ROBBY A. PETROS[2], KESTUR N. AMRUTHESH[1], JYOTI SHAH[1]

[1]Department of Biological Sciences and [2]Department of Chemistry, University of North Texas (UNT), Denton, TX 76203, USA

Systemic acquired resistance (SAR) is a defense mechanism that confers resistance against a broadspectrum of pathogens. SAR is activated systemically through the plant in response to a local infection. The activation of SAR requires long-distance communication by the infected organ with other organs in the plant, resulting in activation of salicylic acid signaling and the simultaneous priming of the uninfested organs to respond faster to any subsequent infections. We have purified a novel abietane diterpenoid, dehydroabietinal, as a SAR inducing factor from the vascular sap-enriched petiole exudates of *Arabidopsis thaliana* leaves infected with a SAR inducing pathogen. Dehydroabietinal, which is systemically translocated through the plant, was active in the picomolar range in activating SAR in Arabidopsis, tomato and tobacco. Our results indicate that the biological induction of SAR by pathogen is associated with the redistribution of dehydroabietinal into a biologically active high molecular weight pool, which is sensitive to trypsin treatment. Future efforts are directed towards understanding dehydroabietinal metabolism and signaling in plants.

P12 GroEL of the aphid endosymbiont *Buchnera aphidicola* is present in the aphid saliva and is recognized by plant defense

RITU CHAUDHARY[1], HAGOP S. ATAMIAN[1], ZHOUXIN SHEN[2], STEVEN P. BRIGGS[2], ISGOUHI KALOSHIAN[1]

[1]Graduate Program in Genetics, Genomics and Bioinformatics, Department of Nematology, University of California, 900 University Ave, Riverside, CA 92521, USA; [2]Division of Biological Sciences, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093, USA

Aphids are major agricultural pests causing vast damage to crops worldwide. Aphids are phloem feeders and harbor the endosymbiont *Buchnera aphidicola* essential for its fecundity ad survival. During feeding, aphids secrete saliva predicted to contain proteins that alter plant defense. Plants have evolved to recognize conserved molecular signatures or microbe-associated molecular patterns (MAMPs) associated with a class of pathogen or herbivore and induce pattern-triggered immunity (PTI). Cell surface localized pattern-recognition receptors (PRR) recognize MAMPs and frequently require the co-receptor BRI1-ASSOCIATED KINASE 1 (BAK1) to trigger PTI. To identify aphid salivary proteins, saliva was collected from 100,000 potato aphids, *Macrosiphum euphorbiae* (Me) and used in mass spectrometry. Among these salivary proteins, were a number of proteins of the endosymbiont *B. aphidicola* origin including the chaperonin GroEL (MeB GroEL). Delivering MeB GroEL into tomato or Arabidopsis through *Pseudomonas syringae* pv*. tomato* type 3 secretion system induced defense against *M. euphorbiae* and *Myzus persicae,* respectively. In addition, MeB GroEL transiently induced oxidative burst and PTI early marker gene expression in Arabidopsis. These MeB GroEL-induced defense responses were BAK1-dependent but independent of the FLAGELLIN-SENSING 2 PRR. Our data indicate that GroEL is an aphid-associated molecular pattern that triggers PTI.

P13 Different contribution of two avirulence effector genes of Japanese *Ralstonia solanacearum* strains to pathogenicity on tobacco

L. CHEN[1], A. KIBA[2], Y. HIKICHI[2], K. OHNISHI[1]

[1]Research Institute of Molecular Genetics, Kochi University, Kochi, Japan; [2]Laboratory of Plant Pathology & Biotechnology, Kochi University, Kochi, Japan

Infection of phylotype-I strains of gram-negative pathogen *Ralstonia solanacearum* on tobacco plants results in different disease symptoms. While strain GMI1000 isolated from South America elicits HR, the mutant lacking two effector genes, *avrA* and *popP1,* causes tobacco plants to wilt. We investigated the involvement of two effector genes of Japanese strains in pathogenicity to tobacco. We used one virulent strain OE1-1, and four HR-eliciting strains, 8107, MAFF211471, MAFF211496, and MAFF301520. There are two types *avrA* sequences, a GMI1000-type and an RS1000-type, in Japanese strains. Both AvrAs are 59% identical in amino acid sequences. While MAFF211496 carries the GMI1000-type *avrA*, other four strains have the RS1000-type *avrA*. Two strains 8107 and MAFF211471 contain *popP1*, however, other three strains have no *popP1* gene. We deleted *avrA* and/or *popP1* genes from HR-eliciting strains. All the mutants, no matter which type of *avrA* gene was deleted, still elicited HR. When *popP1* gene was transferred into the virulent strain OE1-1, the transformant strain significantly reduced the virulence on tobacco. These indicate that *avrA* and *popP1* of Japanese strains have different effects on tobacco wilt incident from those of GMI1000.

P14 Characterization of AvrBsT-elicited immunity in *Arabidopsis*

MI SUN CHEONG, MARY BETH MUDGETT

Department of Biology, Stanford University, 371 Serra Mall, Stanford, CA 94305, USA

Bacteria share common strategies to infect and colonize animal and plant hosts. One is the ability to inject many virulence proteins into their respective host cells by the type III secretion system (TTSS). The central question we investigate is the how plant pathogens use TTSS effectors to alter host physiology to promote disease. The pathogen that we study is *Xanthomonas campestris* pathovar *vesicatoria* (Xcv), the causal agent of bacterial leaf spot disease of tomato, which is endemic in the US and results in significant economic losses worldwide. We have studying the function of AvrBsT, a YopJ-like effector that is widely conserved in plant and animal bacterial pathogens. AvrBsT alters phospholipid signaling in Arabidopsis resulting in the activation of effector-triggered immunity (ETI) in plants lacking the phospholipase SOBER1. AvrBsT physically interacts with a novel microtubule-associated protein (MAP) that is required for ETI. AvrBsT activity results in dynamic changes in the MAP localization during infection. Recent progress in understanding the biochemical and cell biological role of the MAP during plant innate immunity will be presented.

P15 Effector secretion mechanism in the rice blast fungus *Magnaporthe oryzae*

YASIN DAGDAS[1,3], MARTHA C. GIRALDO[2,3], YOGESH K. GUPTA[1], MIHWA YI[2], NICHOLAS J. TALBOT[1,4], BARBARA VALENT[2,4]

[1]School of Biosciences, University of Exeter, EX4 4QD, UK; [2]Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA; [3]Contributed equally to this study; [4]Co-equal corresponding authors

M. oryzae secretes a repertoire of effector molecules which alter host plant metabolism and suppress defence responses. It is known that exocytosis in polarized filamentous fungi happens through the hyphal tip and the secretion of most proteins requires the conventional Endoplasmic Reticulum (ER)-Golgi pathway. It had been thought that the secretion of effectors will follow the same mechanism. Recent studies in *M. oryzae* have, however, shown that symplastic effectors accumulate in a novel sub-apical structure called the Biotrophic Interfacial Complex (BIC), whereas apoplastic effectors outline the invasive hyphae. Secretion from a sub-apical compartment is novel in fungi and how the fungus reorients its secretory apparatus during plant infection is not known. We will show BICs are active secretion sites and Spitzenkorper components are localized at this region. We will provide data to show symplastic and apoplastic effectors of *M. oryzae* are secreted with different mechanisms. Symplastic effectors don't require ER to Golgi pathway; hence they are Brefeldin-A insensitive. Exocyst complex and t-SNAREs on the fungal membrane are required for secretion of symplastic effectors but not for apoplastic ones. Finally we will propose a model to explain reorientation of secretion for effector delivery in *M. oryzae.*

P16 Identification and characterization of host targets of the *Fol* effectors Six1 and Six4

M. DE SAIN, T. O. BOZKURT, J. SKLENAR, P. M. HOUTERMAN, S. KAMOUN, M. REP

Molecular Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, the Netherlands

Plant pathogens secrete effectors to suppress plant defense responses and enhance pathogen growth. On the molecular level, effectors exert their function by manipulating plant targets, often called host or virulence targets. Non-host resistance is probably partially based on the absence of virulence targets. One way to obtain durable resistance is thus to identify these virulence targets and manipulate them in such a way that a host becomes a non-host.
Our research focuses on effectors from the soil-borne fungus *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). Two of the best studied effectors from *Fol* are called Six1 and Six4. Previous research has shown that Six1 is required for full virulence and that Six4 is able to suppress R gene-mediated resistance. Using yeast-two-hybrid and mass spectrometry we have now identified putative virulence targets of these two effectors. We are now trying to validate these targets using pull-down assays and we are investigating their possible role in plant susceptibility.

P17 Cmu1, a secreted chorismate mutase as a tool to elucidate the translocation of effectors into host cells

ARMIN DJAMEI, ANUPAMA GHOSH, REGINE KAHMANN

Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Karl von Frischstrasse 10, Marburg 35043, Germany

A successful colonization of maize by the fungal biotrophic pathogen *Ustilago maydis* requires an effector cocktail in order to suppress plant defense responses and to modulate the host metabolism. The place of action for small secreted effector proteins might be either the biotrophic interface or inside the host cell. The host cell targeting signals and translocation mechanism of fungal effectors is controversially discussed and unclear.
Recently we could show that Cmu1 is an active chorismate mutase that is translocated into the host cells. We try to exploit Cmu1 as a tool to elucidate the translocation mechanism of fungal effectors into host cells. We delete amino acids in Cmu1 which do not perturb the enzymatic activity. These Cmu1 versions are unable to complement the ∆cmu1 phenotype possibly due to the blockage in translocation. Independently several orthologous, Cmu1-like acting fungal chorismate mutases are basis for the identification of conserved aminoacids in for the activity dispensable but for the complementation of ∆cmu1 indispensable regions. Nevertheless the identification of an ultimate translocation motif for host cells awaits further work.

P18 Effector Protein AvrL567 from flax rust modifies cytokinin responses in flax

JEFF ELLIS[1], MARKUS KOECK[2], TONY ASHTON[1], WAN LI[3], SIMON WILLIAMS[3], BOSTJAN KOBE[3], DAVID JONES[2], ADRIENNE HARDHAM[2], PETER DODDS[1]

[1]CSIRO Plant Industry; [2]Australian National University, Canberra, Australia; [3]University of Queensland, Brisbane, Australia

AvrL-567 is a 127 amino acid effector from flax rust with no biological function discernible from its amino acid sequence or structure. It is recognized as an avirulence protein by flax plants carrying the L5, L6 or L7 rust resistance genes by direct interaction between the R proteins and Avr protein. To gain insights into its function during compatible interactions with flax (no L5, L6 or L7 genes present), we performed a yeast two hybrid screen using AvrL567 as bait and as prey, proteins expressed from cDNA from a compatible interaction between flax and rust. One host protein identified in the screen was a cytosolic cytokinin oxidase/dehydrogenase, an enzyme involved in degrading cytokinin to inactive products. Enzyme assays with this host protein indicated that the presence of AvrL567 protein increased its activity. Constitutive expression of AvrL567 in transgenic flax induced several phenotypes in glasshouse grown plants and in tissue culture consistent with cytokinin effects. On the other hand, profound silencing of AvrL567 in transgenic flax rust had no discernible effects on virulence of the rust, possibly because of redundancy in flax rust effector function. The potential reduction of cytokinin in rust infected cells may impact on induction of basal defence via reduction of the cytokinin response regulator protein ARR2, which forms a transcriptional activator of the defence gene PR1 in a ARR2-TGA3 complex (Choi et al., 2010 *Developmental Cell* 19, 284−295).

P19 Quantitative proteomics analysis of the Arabidopsis plasma membrane during immune signaling

J. MITCH ELMORE[1], JUN LIU[1], BRETT PHINNEY[2], GITTA COAKER[1]

[1]Department of Plant Pathology, [2]Genome Center Proteomics Core Facility, University of California at Davis, 210 Hutchison Hall, One Shields Ave. Davis, California, 95616, USA

Many classes of plant pathogens remain outside the host cell membrane during their lifecycle. As a result, the plasma membrane (PM) mediates critical aspects of plant immunity including pathogen recognition, signal transduction, and downstream defense responses. Investigating how the PM proteome changes during these events will lead to a better understanding of plant immune signaling and identify novel components of disease resistance. We have used label-free shotgun proteomics to examine PM dynamics during plant defense signaling. Transgenic *Arabidopsis* plants expressing the bacterial effector AvrRpt2 under the control of a dexamethasone (Dex)-inducible promoter were used to activate the disease resistance protein RPS2 and initiate effector-triggered immunity (ETI). PM vesicles were isolated 6 hours post-Dex treatment and subjected to gel-enhanced liquid chromatography tandem mass spectrometry (Gel LC-MS/MS) for protein identifications. More than 2300 proteins were identified in total and label-free spectral counting was employed to quantify relative protein abundance. A similar approach is being undertaken to examine pattern-triggered immune (PTI) responses upon activation of the FLS2 immune receptor. Preliminary data indicate that activation of ETI and PTI results in distinct, yet overlapping, patterns of PM protein regulation. These experiments provide a framework for understanding global PM proteome dynamics during plant immune responses.

P20 Effectors from diverse pathogens target a limited set of Arabidopsis proteins

P. EPPLE[1,2], N. MCDONALD[1], K. WILEY[1], S. MUKHTAR[1], R. WESSLING[5], R. PANSTRUGA[3], P. BRAUN[4], P. SCHULZE-LEFERT[5], J. DANGL[1,2]

[1]Department of Biology, University of North Carolina at Chapel Hill; [2]HHMI; [3]Department of Biology, RWTH Aachen University, Germany; [4]Department of Plant Systems Biology, Technische Universitaet Muenchen (TUM), Germany; [5]Max-Planck Institute for Plant Breeding Research, Cologne, Germany

The Plant-Pathogen Immune Network-1 (PPIN-1), a Yeast 2-Hybrid based, protein-protein interaction network, describes 165 Arabidopsis proteins targeted by effectors from the evolutionary distant pathogens *Hyaloperonospora arabidopsidis* (*Hpa*) and *Pseudomonas syringae* (*Psy*) (Mukhtar et al., 2011). A subset of these proteins also interact with effectors from *Golovinomyces orontii* (*Go*) and/or *Phytophthora infestans*. We have obtained mutant lines for 124/165 loci and have systematically evaluated these for disease phenotypes after infection with the avirulent *Hpa* isolates Emwa1 and the virulent *Hpa* isolate Noco2. Our results demonstrate that 1) a stringent Y2H assay is a feasible method to identify effector-plant protein interactions, 2) effectors from different pathogens target a limited set of plant proteins repeatedly to fine-tune disease susceptibility and 3) proteins targeted by effectors from at least three different pathogens show a high probability of disease phenotypes when mutated. Additionally, preliminary results indicate that disease phenotypes are not restricted to *Hpa*, but can also be observed after infection with *Psy* or *Go*. Mukhtar et al. (2011) Science 333, 596−601.

P21 Functional analyses of small proteins secreted during infection of Poplar by *Septoria musiva*

A. J. FOSTER, A. SEGUIN, P. TANGUAY

Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., P.O. Box 10380, Stn. Sainte-Foy, Quebec, QC G1V 4C7, Canada

Production in hybrid poplar (*Populus* spp.) plantations can be severely impacted by stem cankers caused by *Septoria musiva*, an aggressive pathogen also responsible for leaf spot. A closely related fungal species, *S. populicola* is not capable of causing stem canker, but it can induce leaf spot disease in poplar. Analysis of the genomes of both species has resulted in the identification of more than 700 putative small secreted proteins, with 88 found to be unique to *S. musiva*. Transcriptional analysis of both fungal species during the infection of leaves and stems of different hybrid clones is currently underway to identify on the small secreted proteins involved in the tree-pathogen interactions. A method for rapid and efficient generation of knockout mutants was developed and is being used to evaluate a subset of putative small secreted proteins, with the goal of identifying effector proteins that are essential for infection. Transcriptional analysis will also be used to examine host gene expression response during infection, by utilizing resistant, partially resistant and susceptible poplar clones.

P22 Biochemical analysis of a *Magnaporthe oryzae* avirulence factor, AVR-Pii

KOKI FUJISAKI, AKIKO ITO, KENTARO YOSHIDA, HIROMASA SAITOH, HIROYUKI KANZAKI, SOPHIEN KAMOUN, RYOHEI TERAUCHI

Department of Genetics and Genomics, Iwate Biotechnological Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan

Avirulence factors of plant fungal pathogens are thought to originally act as effector proteins to manipulate host cell function. We have previously isolated an avirulence factor, AVR-Pii from *Magnaporthe oryzae* (Yoshida et al. 2009). Currently we are studying effector function of AVR-Pii. Live cell imaging showed that AVR-Pii is translocated to inside of rice cells during early infection stage of *M. oryzae*. When AVR-Pii was expressed in rice cells, it accumulated in soluble fraction of cell lysate, and gel filtration analysis showed that AVR-Pii formed two different complexes in the lysate. Further analysis suggested that one form of the complexes was homo-multimer of AVR-Pii, and the other was AVR-Pii-host protein complex. Co-immunoprecipitation (Co-IP) and mass-spectrometry analysis identified two rice Exo70 proteins (OsExo70-1 and OsExo70-2) as candidate interactors of AVR-Pii in rice cells. Exo70 is known as a member of exocyst complex regulating exocytosis pathway in yeast and mammals. In rice, more than 40 members of OsExo70 family are known, although only two of them, OsExo70-1 and OsExo70-2, were identified in this study. Co-IP experiment showed specific interaction between AVR-Pii and OsExo70-1 but not other OsExo70 members, suggesting that AVR-Pii specifically targets OsExo70-1 and OsExo70-2.

P23 Scanning Electron Microscopy of *Plasmopara halstedii* infection during sunflower downy mildew and inventory of pathogenicity effectors towards definition of avirulence genes

QUENTIN GASCUEL, MAGALIE PICHON, ERIKA SALLET, NICOLAS BLANCHET, MARIE-CLAUDE BONIFACE, PATRICK VINCOURT, LAURENCE GODIARD

Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, INRA, CNRS, CS52627, F-31326 Castanet-Tolosan, France

Downy mildew in sunflower (*Helianthus annuus*) is caused by the biotroph oomycete *Plasmopara halstedii*. The emergence of 17 new virulent races in 20 years in France leads to the break-down of many *Pl* resistance genes used in sunflower hybrids. Understanding the rapid emergence of new virulence profiles is a prerequisite for defining plant sustainable resistance. Massive cDNA sequencing allowed the identification of 20 putative *P.halstedii* RXLR and CRN pathogenicity effectors (As-sadi et al., BMC Genomics 2011). The genome sequencing of 7 races of *P.halstedii* should help us in defining the repertoire of CRN and RXLR effectors. Presence-absence, polymorphism studies among races and *in planta* time-course expression of putative effectors are used to select the best candidate avirulence genes. Their expression will be tested in sunflower using an infiltration method developed in our laboratory with LBA4404 *A. tumefaciens* strain. Over-expression of 5 putative CRN effectors, using this method, already suggests that some of them are recognized *in planta.* In addition, histological aspects of the infection process of sunflower by *P.halstedii* are being investigated with Scanning Electron Microscopy (SEM) in order to select the critical steps of the interaction in resistant and susceptible sunflower lines.

P24 Secretion of *Magnaporthe oryzae* cytoplasmic effectors occurs by a Golgi-independent mechanism during biotrophic invasion of rice

MARTHA C. GIRALDO, YASIN DAGDAS, YOGESH K. GUPTA, MIHWA YI, NICHOLAS J. TALBOT, BARBARA VALENT

Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA; School of Biosciences, University of Exeter, EX4 4QD, UK

Rice blast disease, *Magnaporthe oryzae*, in its biotrophic phase secretes cytoplasmic effectors and apoplastic effectors. Cytoplasmic effectors preferentially accumulate in the biotrophic interfacial complex (BIC), a novel in planta structure that locates beside the tip of the initially filamentous invasive hypha and remains beside the first differentiated bulbous invasive hypha cell. In contrast, apoplastic effectors uniformly surround the invasive hyphae that grow to fill the invaded cell. We report live cell microscopy of invasive hyphae expressing various fluorescent secretion machinery components and various fluorescent effectors. Localization of Spitzenkörper and polarisome markers confirmed distinct growth and secretion patterns for the filamentous and bulbous invasive hyphae, and suggested that secretion into BICs continued while invasive hyphae grew elsewhere in the host cell. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A (BFA) treatment blocked secretion of apoplastic effectors, which were retained in the ER, but not secretion of cytoplasmic effectors. Fluorescence Recovery After Photobleaching experiments confirmed that cytoplasmic effectors continued to accumulate in BICs in the presence of BFA. Pathogen mutants that failed to express either exocyst complex components or a t-SNARE were defective in secretion of BIC-localized effectors, but not apoplastic effectors. We propose a model in which exocyst and SNARE complexes play a role in the secretion of cytoplasmic effectors into BICs by an unconventional, Golgi-independent secretory pathway.

P25 Phosphorylation of HopQ1, a Type III Effector from *Pseudomonas syringae,* creates a binding site for host 14-3-3 proteins

FABIAN GISKA[1], MALGORZATA LICHOCKA[1], MARCIN PIECHOCKI[1], MICHAL DADLEZ[1], ELMON SCHMELZER[2], JACEK HENNIG[1], MAGDALENA KRZYMOWSKA[1]

[1]*Institute of Biochemistry and Biophysics, Pawinskiego 5a, 02-106 Warsaw, Poland*; [2]*Max-Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Germany*

HopQ1, a TTSS effector secreted by *Pseudomonas syringae* pv. *phaseolicola*, promotes the development of halo blight in bean. However, when this same effector is injected into tobacco cells, it is recognized by the immune system and prevents infection. Although the ability to synthesize HopQ1 determines host specificity, the role it plays inside plant cells remains unexplored. Following expression *in planta*, HopQ1 was shown to co-purify with host 14-3-3 proteins. The physical interaction between HopQ1 and 14-3-3a was confirmed using FRET-FLIM techniques. Mass spectrometric analyses detected specific phosphorylation of the canonical 14-3-3 binding site present in HopQ1. Substitution within this motif abrogated the interaction and led to altered subcellular localization of HopQ1. Moreover, the mutated HopQ1 protein showed reduced stability *in planta*. These data suggest that the association between host 14-3-3s and HopQ1 is important for modulating the virulence properties of this bacterial effector.

P26 Dissecting the molecular events during effector recognition by the Arabidopsis resistance protein RPP1

SANDRA GORITSCHNIG[1], ADAM D. STEINBRENNER[1], KSENIA V. KRASILEVA[1,2], KARL SCHREIBER[1], BRIAN J. STASKAWICZ[1]

[1]Department of Plant and Microbial Biology, University of California Berkeley, 111 Koshland Hall, Berkeley, CA 94720, USA; [2]current address: Department of Plant Sciences, University of California Davis, USA

Effector-triggered immunity is the plant's main defense mechanism against adapted pathogens, during which resistance (R) proteins recognize the presence of cognate pathogen-delivered effector proteins and trigger a potent immune response. The *Arabidopsis thaliana* R protein RPP1-WsB recognizes several alleles of the effector ATR1 from *Hyaloperonospora arabidopsidis*. A direct interaction between the R protein and the recognized effector is supported by *in planta* association of the proteins as well as mutational analyses of both ATR1 and RPP1, which have identified individual residues that alter recognition specificity. Here we present our current studies aiming at gaining a better understanding of the events involved in RPP1 activation upon ATR1 recognition. The RPP1 protein contains a predicted myristoylation site (G2) as well as a functional nuclear localization sequence (NLS). We are using stably transformed transgenic Arabidopsis to ascertain the subcellular localization of RPP1 prior to and upon ATR1 recognition. Introducing mutations at G2 and the NLS provides means to investigate the role of sub-cellular partitioning in RPP1 function. Furthermore, we are seeking to identify additional interacting proteins of RPP1 and/or ATR1 during the recognition event in order to further dissect the molecular events involved in R protein activation by a directly associating effector protein.

P27 Neo-functionalisation of SUMO paralogs contributes to plant innate immunity

VALENTIN HAMMOUDI, M. J. MAZUR, G. VLACHAKIS. H. A. VAN DEN BURG

University of Amsterdam, Molecular Plant Pathology, Amsterdam, The Netherlands

As activation of defenses is an important energy cost, plants have to fine tune defense gene regulation. Hence, non-infected plants likely repress innate immunity, while under biotic stress they can rapidly activate defense-associated transcriptional reprogramming. Interestingly, the SUMO machinery, including the SUMO E3 ligase SIZ1, suppresses the Salicylic Acid (SA)-dependant defense responses. The Arabidopsis genome contains 8 putative SUMO genes. The sumo1; sumo2 double mutant phenocopies the constitutives defense activation seen in siz1-2 mutants. Hence, SIZ1-dependent conjugation of SUMO1/2 to unknown targets controls SA signaling. We examine the role of a divergent paralog: SUMO3, which emerged from a SUMO2 gene duplication specific to Brassicaceae. SUMO3 rapidly diverged from the core SUMO1/2 genes, displaying differences in gene expression. Expression of SUMO3 is SA-dependent and SUMO3 does not influence SUMO1/2 conjugation homeostasis. Contrary to SUMO1/2, we have shown that SUMO3 positively contributes to plant defenses promoting resistance against virulent *Pseudomonas*. This constitutes the first example of neo-functionalisation of SUMO paralogs in plants.

P28 The Arabidopsis lectin receptor kinase LecRK-I.9 confers late blight resistance in Solanaceous plants

M.HAN[1], R. BLANCO-PORTALES[3], E. VAN DER VOSSEN[3], F. GOVERS[1,2], K. BOUWMEESTER[1,2]

[1]*Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands;* [2]*Centre for BioSystems Genomics (CBSG), Wageningen, The Netherlands;* [3]*Laboratory of Plant Breeding, Wageningen University, Wageningen, The Netherlands*

Plant receptor kinases are pivotal in sensing signals that are released upon pathogen invasion, and to subsequently initiate defense responses. Previously, we showed that the Arabidopsis lectin receptor kinase LecRK-I.9 functions in Arabidopsis resistance to the oomycete plant pathogen *Phytophthora brassicae* (Bouwmeester *et al*., 2012 PLoS Pathogens). In this study, we investigated whether ectopic expression of *LecRK-I.9* in Solanaceous plants could confer enhanced resistance to the late blight *pathogen Phytophthora infestans. In order to test this, transgenic potato plants stably expressing LecRKI. 9* were generated and tested in disease assays for altered resistance towards *P. infestans*. Similarly, *N. benthamiana* plants that transiently express *LecRK-I.9* were subjected to *P. infestans*. In both cases, the plants were less susceptible to *P. infestans*. *LecRK-I.9* expression did not lead to enhanced resistance towards with the fungal pathogen *Botrytis cinerea*. The obtained results show that *LecRK-I.9* specifically confers enhanced resistance to *P. infestans* in both potato and *N. benthamiana*, and hence provides a potential strategy to acquire *Phytophthora* resistance in Solanaceous crop plants.

P29 The *Ustilago* effector Pep1 and its role in biotrophy of smut fungi

C. HEMETSBERGER[1], C. HERRBERGER[1], B. ZECHMANN[1], M. HILLMER[1], M. THINES[2], G. DOEHLEMANN[1]

[1]*Dept. for Organismic Interactions, Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str. 10, 35043 Marburg, Germany;* [2]*Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt am Main, Siesmayerstr. 70, 60323 Frankfurt, Germany*

The secreted fungal effector Pep1 is essential for successful penetration of the host epidermis and establishment of biotrophic interaction in the *Ustilago maydis* / maize pathosystem as well as in the related covered smut of barley, *Ustilago hordei*. Deletion of *pep1* does not affect saprobic growth, however pathogenic development is arrested after the penetration attempt and a strong plant immune response is elicited. Recently, we could show that Pep1 acts as inhibitor for plant peroxidases and therefore has the ability to suppress the oxidative burst, the primary immune response of the host plant. This enables colonization of the host tissue by the fungus. Another interesting observation was the functional conservation of Pep1 among the smut fungi *U. maydis* and *U. hordei* was found. To further investigate the conservation of Pep1 in different pathogens, related smut species were analyzed for occurrence of the *pep1* gene. This revealed a remarkable degree of sequence conservation of *pep1* in pathogen infecting monocotyledonous as well as dicotyledonous host plants, indicating that this effector carries a fundamental mechanism in virulence of biotrophic smuts. Currently, complementation assays are being carried out to verify functional preservation of different *pep1* orthologues.

P30 Regulation of intracellular redox by glyceraldehydes-3-phosphate-dehydrogenase during plant innate immunity

ELIZABETH HENRY, JUN LIU, J. MITCH ELMORE, GITTA COAKER

Department of Plant Pathology, University of California at Davis, Hutchison Hall 210/201, One Shields Ave., Davis, CA 95616, USA

Glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) are important enzymes with diverse cellular regulatory roles in vertebrates including regulation of reactive oxygen species (ROS), but few reports have investigated GAPDH importance outside of their role in glycolysis in plants. We have found that GAPDHs are upregulated during effector triggered immunity at the protein level. A genetic approach was used to investigate the importance of different GAPDH members during plant innate immune responses using the interaction between *Arabidopsis thaliana* and the bacterial plant pathogen *Pseudomonas syringae* pv. *tomato* (*Pto*). A subset of GAPDH mutants exhibit enhanced disease resistance phenotypes. These mutants show accelerated programmed cell death and increased electrolyte leakage in response to avirulent pathogen recognition. Characterization of ROS production in some GAPDH knockout lines showed increased ROS production in response to stress elicitors. Additionally, one GAPDH isoform dynamically re-localizes to the site of ROS production during defense responses. These results indicate a role for GAPDHs in cellular redox regulation during plant immune responses against microbial pathogens. ROS are important intra-and intercellular signaling molecules, essential in resistance against many types of pathogens. Understanding mechanisms of ROS flux regulation by proteins like GAPDHs is important in elucidating downstream signaling events during innate immune responses in plants.

P31 Cell death suppression during the interaction of *Ustilago* and barley

ALEXANDER HOF[1], DANIEL MATHOW[1], DANIELA AßMANN[1], DANIELA SCHWAMMBACH[1], RUTH EICHMANN[2], RALPH HÜCKEHOVEN[2], GUNTHER DOEHLEMANN[1]

[1]*Max Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl-von-Frisch-Str. 10, D-35043 Marburg, Germany;* [2]*Center of Life and Food Science Weihenstephan, Technische Universitaet Muenchen, Emil-Ramann-Str. 2, D-85350 Freising-Weihenstephan, Germany*

In plants programmed cell death (PCD) is an essential defense mechanism during pathogen attack, making PCD prevention essential to biotrophic plant pathogens such as *Ustilago hordei.* During the compatible interaction of this basidiomycetous fungus with its host plant barley, PCD is fully prevented, whereas deletion mutants of the secreted effector protein Pep1 cause PCD comparable to the non-host resistance reaction after infection with *U. maydis.* Microscopical analyses revealed that plants overexpressing the conserved cell death suppressor Bax Inhibitor-1 (BI-1) show an increased susceptibility to the non-host pathogen *U. maydis*. Interestingly, BI-1 seems to play no role in the interaction with the *U. hordei* or *U. maydis pep1* deletion mutant, respectively, indicating that the induced cell death reaction by the effector mutant is mediated by a BI-1 independent pathway. Our results point towards a role of apoptosis-like cell death during non-host responses, while autophagy might be induced during interactions independent from BI-1. With a combination of microscopic and enzymatic approaches, we are dissecting the different modes of programmed cell death triggered by the described fungal strains.
Additionally, microarray experiments have provided new insights into the transcriptome of *U. hordei*, revealing a phase specific regulation of secreted effectors. Candidates for cell death suppressing proteins will be used in different screening approaches for which recent progress will be presented.

P32 Activities of Serine hydrolases during the interaction of *Nicotiana benthamiana* with *Pseudomonas syringae*

TRAM NGOC HONG, RENIER VAN DER HOORN

The Plant Chemetics Lab, Max Planck Institute for Plant Breeding Research, Carl-von-linne-weg 10, 50829 Cologne, Germany

Serine Hydrolases (SHs) play crucial roles in plant immunity. By applying activity-based protein profiling using flourophosphonate-based probes, we investigate differential of SH activities of *Nicotiana benthamiana* during the interactions with *Pseudomonas syringae*. The infections involve *Pseudomonas syringae* pv. *tomato* DC3000 and the derived mutants to study PTI, ETI, and ETS. Furthermore, the common and strain-specific infection strategies are studied with three different pathovars (*tabaci, syringae, tomato)*. We found several differentials in activities of SHs during this interaction. In order to improve the separation of the signals, sub-proteome fractions such as apoplastic fluid, membrane and nuclei are analyzed, and two dimensional protein gel electrophoresis is performed. The differentials are identified by mass spectrometry, and studied further to elucidate the underlying molecular mechanism.

P33 The role of papain-like cysteine proteases of tomato in pathogen defense

MUHAMMAD ILYAS[1], TOLGA BOZKURT, SOPHIEN KAMOUN[2], RENIER VAN DER HOORN[1]

[1]Plant Chemetics Lab, Max Planck Institute for Plant Breeding Research, Carl-von-Linne Weg 10, 50829 Cologne, Germany; [2]The Sainsbury Laboratory, John Innes Centre, Norwich, NR4 7UH, UK

Tomato creates a proteolytic apoplast during defense by secreting a series of proteases, including cysteine proteases PIP1 and Rcr3. The Avr2 from *Cladosporium fulvum* inhibits the cysteine proteases Rcr3 and PIP1. Rcr3 is required for *Cf2*-mediated perception of Avr2. This indirect perception mechanism is consistent with the guard model, which predicts that Rcr3 is a virulence target of Avr2, guarded by the *Cf2* resistance gene product. The higher abundance of PIP1 compared to Rcr3, however, suggests that Rcr3 is rather a decoy that only functions in Avr2 perception. Here we show that silencing of *PIP1* in the transgenic tomato enhances *C. fulvum* susceptibility, whereas *rcr3* mutant plants do not show increased susceptibility in the absence of *Cf2*, consistent with the decoy model. Rcr3 and PIP1 are also inhibited by Epic1 and Epic2B, secreted by *Phytophthora infestans* during infection. Epics are not recognized through the Rcr3-Cf2 perception system, but *Cf2/rcr-3-3* mutant plants are more susceptible for *P. infestans* when compared to *Cf2/Rcr3* plants, suggesting a role for Rcr3 in *P. infestans* resistance in the absence of Epic recognition. We now found that *Cf0/rcr3-3* mutant plants are also more susceptible to *P. infestans* when compared to *Cf0/Rcr3* plants indicating a role for Rcr3 in the absence of Cf2 in *P. infestans* resistance.

P34 Abiotic and biotic stress responses and energy metabolism are targets of *Psuedomonas syringae* virulence effector HopI1

JOANNA JELENSKA, JEAN T. GREENBERG

Department of Molecular Genetics and Cell Biology, University of Chicago, 929 E 57th St, Chicago, IL 60637, USA

Pseudomonas syringae injects multiple type III effectors to plant cells for a successful infection. We previously characterized the HopI1 effector that suppresses salicylic acid (SA) and related defenses in chloroplasts and disturbs thylakoid structure. HopI1 acts as a co-chaperone of Hsp70 by binding Hsp70 through its J domain and increasing Hsp70 ATP hydrolysis activity. HopI1 induces cytosolic Hsp70 that is required for its virulence function and renders plants heat tolerant, while high temperature complements the growth defect of Δ*hopI1* strain. Here we show that HopI1 directly targets SA synthesis and energy metabolism. Upon activation of SA production, ICS1, an enzyme in SA biosynthesis pathway, coimmunoprecipitates with HopI1 and Hsp70. Further analysis of HopI1 complexes identified chloroplast ATP synthase as another client of HopI1/Hsp70. Plants expressing HopI1 have reduced levels of ATP synthase and lower starch content. HopI1-induced changes in thylakoid structure probably occur due to reduced ATP synthase levels. Since a fungal virulence toxin also targets ATP synthase, it is likely that it plays a defense-promoting role. We hypothesize that HopI1 uses Hsp70 to suppress chloroplast-based defenses by disrupting ICS1 complexes and disassembling multisubunit ATP synthase or otherwise affecting these proteins' maturation, stability, and/or degradation.

P35 Cereal rust (and smut) effectors promote disease susceptibility

DAVID L. JOLY[1,2], XIAO SONG[1], PATRICK GANNON[1], GUUS BAKKEREN[1], BARRY J. SAVILLE[2]

[1]*Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, PO BOX 5000, Summerland, BC V0H 1Z0, Canada;* [2]*Forensic Science Department, Trent University, Peterborough, ON K9J 7B8, Canada*

The efficiency of surrogate bacterial delivery systems to assess the virulence functions of fungal and oomycete effectors have been well established in *Arabidopsis* (using *Pseudomonas syringae* pv. *tomato*). However, such heterologous systems have not been optimized for use in other pathosystems. We are developing a surrogate system to dissect the interaction between the cereal crops wheat and barley and their rust and smut pathogens. Such assays will be salutary to the molecular dissection of these interactions in which obligate biotrophic lifestyles and complex lifecycles preclude traditional genetic approaches. The recent and ongoing genomic analyses of *Ustilago hordei* (barley covered smut) and *Puccinia triticina* (wheat leaf rust) uncovered hundreds of candidate effectors. Using comparative genomic and transcriptomic approaches, we selected interesting candidate rust (and smut) effectors and explored their ability to promote disease susceptibility in cereal-adapted *Pseudomonas* and *Xanthomonas* species. Preliminary results suggest that many of the tested effectors conferred increased bacterial growth. In addition, candidate effectors were also tested for their potential to trigger incompatibility through avirulence functions using the developed system, as well as using *Pseudomonas fluorescens* 'EtHAn' as the delivery system.

P36 Exploiting the potato NB-LRR gene family to improve genome annotations and to identify novel functional resistance genes

FLORIAN JUPE[1,2], WALTER VERWEIJ[2], LEIGHTON PRITCHARD[3], GRAHAM J. ETHERINGTON[2], GLENN J. BRYAN[1], JONATHAN D.G. JONES[2], INGO HEIN[1]

[1]Cell and Molecular Sciences, The James Hutton Institute (JHI), Dundee, DD2 5DA, UK; [2]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK; [3]Information and Computational Sciences (JHI)

Plant pathogen effector proteins are typically recognised by plant resistance (*R*) genes. A key class of *R* genes contain a nucleotide-binding and leucine-rich repeat domain and are collectively known as NB-LRRs. As part of an effort to accelerate the process of functional *R* gene isolation, we have performed an exhaustive amino-acid motif based search of the published and annotated potato genome from the doubled-monoploid clone DM1-3_516_R44 and identified 438 NB-LRR type genes (Jupe et al., 2012). We have exploited the *R* gene sequence information and designed a NB-LRR capture platform that, in conjunction with second generation sequencing technology can be used to aid the annotation of related genomes and to identify and clone functional NB-LRR genes from wild *Solanum* species. This approach was successfully tested on the sequenced potato clone and facilitated the discovery of 338 additional NB-LRR encoding genes from un-annotated regions. Physical map positions were established for 635 predicted NB-LRR genes across all 12 potato chromosomes. The majority of NB-LRRs are physically organised within 85 clusters. Importantly, this approach has successfully been applied to identify and map functional NB-LRR genes from diploid *Solanum* species.

P37 The role of conserved *Phytophthora* effectors in virulence

JULIETTA JUPE, J. MORRIS, P. HEDLEY, E. HUITEMA

College of Life Sciences, Division of Plant Sciences, University of Dundee at JHI, Errol Road, Invergowrie, DD2 5DA, UK

Pathogen-host interactions feature a dynamic interplay between defence signalling cascades and specialized pathogen machineries that can subvert immunity. *Phytophthora capsici* affects a wide range of important crop families such as cucurbits and solanaceae. Despite the presence of diverse effector repertoires, *P. capsici* shares a number of conserved RXLR proteins with other *Phytophthora* members in this genus. This suggests that conserved effectors play important roles in virulence and form attractive targets to disable pathogenesis. To understand the role of those effector proteins in *Phytophthora* parasitism, we explored the infection process of *P. capsici* on *Solanum lycopersicum*.
We found that infection features a biotrophy phase followed by necrotrophy, lesion development and sporulation. A microarray experiment was conducted to assess gene expression for both *P. capsici* and *S. lycopersicum* gene models during infection. We identified 3691 differentially expressed genes for *P. capsici* and 4082 for *S. lycopersicum*. Analyses of 73 differentially expressed RXLR coding genes, revealed distinct expression patterns for spores, early biotrophy and biotrophy. Furthermore, Y2H screens and confocal microscopy indicate conserved functions for a number of RXLR effectors that are conserved within *Phytophthora* sp. These analyses will allow us to assess the roles of effector proteins in virulence and to obtain conceptual explanations of host specificity, pathogen ingress and defence suppression.

P38 Arms race co-evolution of *Magnaporthe oryzae AVR* gene and rice *R* genes driven by their physical interactions

HIROYUKI KANZAKI, KENTARO YOSHIDA, HIROMASA SAITOH, KOKI FUJISAKI, AKIKO HIRABUCHI, RYOHEI TERAUCHI

Department of Life Science, Iwate Biotechnology Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan

Between pathogen and host, antagonistic interactions impose strong reciprocal selection on each organism, leading to the development of arms race evolutionary dynamics. However, studies on specific recognition and co-evolution between resistance (*R*-) gene and avirulence (*AVR*-) gene are still limited. Here we show that *AVR-Pik* of *Magnaporthe oryzae*, the rice blast pathogen, and cognate rice *R*-gene *Pik* exhibit high levels of amino acid changes. We found a tight recognition specificity of *AVR-Pik* alleles (*Pik/km/kp/k*-) by different *Pik* alleles (*AVR-Pik-A/C/D/E*). Pik is composed of two proteins, Pik1 and Pik2, both consisting of coiled-coil - nucleotide binding site - leucine-rich repeat (CC-NBS-LRR) domains. We found that AVR-Pik physically interacts the N-terminal CC domain of Pik1 in yeast 2-hybrid assay as well as in *in-planta* co-immunoprecipitation assay. Furthermore, this binding specificity mainly determines the recognition specificity between AVR-Pik and Pik alleles. These data suggest that the physical interaction results in the arms race co-evolution between *AVR-Pik* and *Pik*.

P39 *Xanthomonas* type III effector XopD desumoylates tomato transcription factor SlERF4 to suppress ethylene responses and promote pathogen growth

JUNG-GUN KIM, WILLIAM STORK, MARY BETH MUDGETT

Department of Biology, Stanford University, Stanford, CA 94305-5020, USA

XopD is a SUMO protease from *Xanthomonas campestris* pathovar *vesicatoria* (Xcv) required for pathogen growth and delay of symptom development in tomato. XopD also contains two EAR motifs implicated in ethylene (ET) signaling and transcriptional repression. Here we discovered that XopD targets SlERF4, a tomato ET responsive transcription factor to inhibit ET-stimulated immunity. ET levels and biosynthesis mRNAs were significantly higher in Xcv *ΔxopD*-infected leaves compared to Xcv-infected leaves. Both ET production and perception were required for Xcv immunity and symptom development. Furthermore, *SlERF4* mRNA expression was required for Xcv *ΔxopD*-induced ET production and ET-stimulated immunity. XopD was found to colocalize with SlERF4 in subnuclear foci and hydrolyze tomato SUMO1 from K53 of SlERF4 resulting in SlERF4 destabilization. Mutation of K53 to R53 prevented SlERF4 sumoylation, decreased SlERF4 levels, and reduced SlERF4 transcription. We conclude that XopD directly targets and desumoylates SlERF4 to repress ET induced-transcription required for Xcv immunity.

P40 Altering the recognition specificity of an NB-LRR protein by engineering its 'Guardee'

SANG HEE KIM, ROGER W. INNES

Department of Biology, Indiana University, Myers Hall 302, 915 East Third Street, Bloomington, IN 47405, USA

Pathogen recognition by NB-LRR proteins is often accomplished by an indirect mechanism in which the NB-LRR protein senses modification of other host proteins by pathogen effectors. How NB-LRR proteins sense such modifications is poorly understood. To gain insight into this process we have focused on RPS5, an NB-LRR protein from Arabidopsis, and AvrPphB, a cysteine protease secreted by *Pseudomonas syringae.* RPS5 senses the presence of AvrPphB by monitoring the status of PBS1, a protein kinase that is cleaved by AvrPphB in the middle of its activation loop. Here we show that the requirement for cleavage of PBS1 can be bypassed by inserting from three to seven amino acids into the PBS1 activation loop, indicating that RPS5 recognizes a subtle conformational change in PBS1, rather than cleavage. In addition, we replaced seven amino acids flanking the AvrPphB cleavage site with the recognition sequence for a different cysteine protease, AvrRpt2. This modified PBS1 did not activate RPS5 by itself, but did activate RPS5 in the presence of AvrRpt2, demonstrating that the recognition specificity of RPS5 can be switched by making a small modification in PBS1. Engineering of NB-LRR 'guardees' thus provides a new strategy for broadening NB-LRR specificity.

P41 LysM effectors of fungal plant pathogens contribute to virulence in various manners

ANJA KOMBRINK, JASON J. RUDD, BART P.H.J. THOMMA

Laboratory of Phytopathology, Wageningen University, 6708 PB Wageningen, Netherlands

LysM effector genes are found in the genomes of a wide range of fungal species. LysM effectors are secreted proteins that contain a varying number of LysM domains and no other recognizable protein domains. LysM domains are carbohydrate-binding modules that occur in various proteins that are produced by a variety of organisms. Ecp6 is the first characterized LysM effector that was isolated from the tomato leaf mould fungus *Cladosporium fulvum* and that is instrumental for fungal virulence. Carbohydrate binding assays demonstrated that Ecp6 specifically binds chitin, the major constituent of fungal cell walls that acts as microbial-associated molecular pattern (MAMP) that triggers immune responses upon recognition by the host. We demonstrated that the chitin-binding effector Ecp6 can compete with plant receptors for chitin binding, and thus prevents the activation of immune responses. Two orthologues of Ecp6 were identified in the fungal wheat pathogen *Mycosphaerella graminicola*, of which one suppresses chitin-induced immune responses in a similar fashion as Ecp6. Interestingly, unlike Ecp6, both *M. graminicola* LysM effectors were able to inhibit degradation of fungal hyphae by plant chitinases. Many fungal genomes carry multiple LysM effector genes that share only low sequence conservation and encode varying LysM domain numbers per molecule. Therefore, we hypothesize that different fungal LysM effectors are likely to bind different carbohydrate substrates, exert other functions, or are active in other stages of the fungal life cycle than plant infection. We will report on our most recent findings on LysM effector substrates and functions.

P42 Potential pathogen targets and innate immunity genes of durum wheat

K. V. KRASILEVA[1], B. SCHWESSINGER[2], V. BUFFALO[1], J. DUBCOVSKY[1]

[1]Department of Plant Sciences & [2]Department of Plant Pathology, University of California, One Shield Ave, Davis, CA 95616, USA

Wheat (Triticum sp.) comprises several diploid and polyploid species. Durum wheat, whose grain is commonly used in pasta and couscous, has a tetraploid genome of AABB structure. The closet diploid relative of AA genome is a non-domesticated *Triticum urartu*. As part of a wider program to establish genomic tools in wheat, we sequenced and assembled transcriptomes of durum wheat, *Triticum turgidum* subsp. *durum*, and *Triticum urartu* and surveyed both transcriptomes for genes involved in plant immunity and susceptibility to pathogens. Both wheat species contain an explosion of classical NBS-LRR disease resistance genes, 1125 in *T. urartu* and 2040 in *T. turgidum*. Interestingly, the downstream signaling components of NBS-LRRs identified in *Arabidopsis* are conserved in wheat. As forward genetic analyses in wheat are complicated by polyploidy, comparing disease resistance pathways across species will lead to focused investigation of wheat immunity.

P43 Host-adapted colonization strategies of the root symbiont *Piriformospora indica*

U. LAHRMANN, Y. DING, A. ZUCCARO

Max Planck Institute for Terrestrial Microbiology, Organismic Interactions, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany

Piriformospora indica is a mutualistic endophyte that stimulates growth, alleviates salt stress and induces systemic resistance to pathogens in different hosts. This fungus establishes a long lasting interaction with the roots of both, the agronomically important monocot barley and the dicot model plant Arabidopsis. We hypothesized that successful colonization of different plants would require host-specific fungal gene expression and documented these differences by a global characterization of fungal transcriptional responses to barley and Arabidopsis at different symbiotic stages. We show here that *P. indica* colonization of barley roots is characterized by a dramatic change in hyphal morphology that is associated with an abrupt rearrangement of the fungal metabolism to a saprophytic nutrition at later stages. During interaction with Arabidopsis, on the other side, this symbiont is able to undergo a longterm feeding relationship with living cortex cells. This data are supported by microscopy and colonization analysis of host and fungal mutants and provides strong initial evidence for host-adapted fungal colonization strategies in broad-host root endophytes.

P44 Pathogenic genetic array of bacterial effectors to screen for conserved eukaryotic pathways

A. H. LEE, MAGGIE MIDDLETON, JI-YOUNG YOUN, SARA SHARIFPOOR, BRENDA ANDREWS, DARRELL DESVEAUX, DAVID GUTTMAN

Cell & Systems Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario, M5S3B2, Canada; Donnelly Centre for Cellular & Biomolecular Research, University of Toronto, 160 College Street, Toronto, Ontario, M5S3E1, Canada

Successful bacterial pathogens attack key intracellular host processes to promote their survival. One mechanism used by bacteria to achieve this is by injecting virulence factors (effectors) directly into the host cell. While we know the identity and general activity (e.g. suppression of defense signaling) of many effectors, very few have well-characterized enzymatic activities or host targets. To overcome the challenges of functional characterization of effectors, we designed a heterologous high-throughput screen by expressing bacterial effectors in the budding yeast, *Saccharomyces cerevisiae*. Bacterial effectors that altered yeast fitness are expected to effectively target yeast cellular processes, which can be subsequently identified via a modified synthetic genetic array (SGA). Using this method, we screened the viable yeast haploid deletion strain collection for mutants that suppress or enhance lethality of bacterial effector expression. By applying a genome-wide approach, we have identified genetic interactors of the *Pseudomonas syringae* effector HopZ1a to be enriched in cytoskeletal processes, which is congruent with its ability to alter the plant microtubule network. Using a similar approach, we have potentially uncovered novel cellular processes targeted by two additional *P. syringae* effectors, HopF2 and HopX1.

P45 Identification of RIN4 protein complexes in Arabidopsis by Blue Native PAGE

D.-H. LEE, G. COAKER

Department of Plant Pathology, University of California, Davis, Hutchison Hall 210, One Shields Avenue, Davis, CA, 95616 USA

Protein complexes play important roles in pathogen recognition and innate immune signaling. The Arabidopsis protein RIN4 is a crucial regulator of plant immunity, which interacts with various pathogen effectors as well as immune receptors. In order to elucidate RIN4-associated immune complexes in Arabidopsis, we performed BlueNative polyacrylamide gel electrophoresis (BNPAGE) and detected three major RIN4-associated protein complexes in microsomal fractions, including 1000kDa, ~400kDa, and ~200kDa complexes. In the major RIN4 complexes, the biggest complex contains the plant NLR immune receptors RPM1 and RPS2. In addition, the presence of RPS2 was confirmed in the 1 MDa RIN4-complex through two-dimensional BNPAGE.
These results indicate that RIN4 and NLR immune receptors are present in large, membrane localized supercomplexes. Future investigations will focus on identification of individual complex constituents and dynamic changes in complexes during immune perception.

P46 HopZ3, a *P. syringae* type III effector, is an active acetyl-transferase

JIYOUNG LEE, JEAN T. GREENBERG

Molecular Genetics and Cell Biology, The University of Chicago, USA

A successful strategy used by many bacterial pathogens of both plants and animals is to use a type III secretion system to deliver their effector proteins into host cells to promote disease. *Pseudomonas syringae* pv *syringae* B728a, a pathogen of bean and *Nicotiana benthamiana* has a strong epiphytic life style. One effector in particular, HopZ3, restricts epiphytic growth on *N. benthamiana*, but promotes epiphytic survival on tomato and Arabidopsis. An emerging theme is that some type III effectors are enzymes that modify host targets and have autocatalytic activity. HopZ3 belongs to the YopJ-like family of proteins that shows acetyltransferase activity. We found that HopZ3 acetylates itself in vitro and mapped an acetylation site in the protein. Mutation of this site impaired the ability of HopZ3 to fully complement a *Psy*B728a ΔhopZ3 mutant. We identified a complex that is targeted by HopZ3 and found that many, possibly all members of the complex are modified by HopZ3 and affected by acetylation. Our data highlight the importance of posttranslational modifications of and by effectors.

P47 A novel host signaling protein is required for the recognition of the type III secreted effector HopZ1a

J. D. LEWIS[1,2,3], J. WAN[1], D. S. GUTTMAN[1,4], D. DESVEAUX[1,4]

[1]Department of Cell & Systems Biology, 25 Willcocks St., University of Toronto, Toronto, ON, M5S 3B2, Canada; [2]Plant Gene Expression Center, United States Department of Agriculture, 800 Buchanan St., Albany, CA, 94710, USA; [3]Department of Plant & Microbial Biology, University of California Berkeley, 800 Buchanan St., Albany, CA, 94710, USA; [4]Center for the Analysis of Genome Evolution and Function, 25 Willcocks St., University of Toronto, Toronto, ON, M5S 3B2, Canada

The plant pathogen *Pseudomonas syringae* causes disease in more than 100 plant species using the type III secretion system to secrete and translocate effector proteins into the plant. Many of these effector proteins are believed to function primarily in the suppression of host defense signaling. However recognition of these effector proteins by resistance (R) proteins induces a defense response. The YopJ/HopZ family of effector proteins is evolutionary diverse and found in both animal and plant pathogens. We previously demonstrated that HopZ1a elicits effector triggered immunity, when it is recognized in *Arabidopsis* by the ZAR1 R protein. However, recognition of HopZ1a does not require any known defense-related proteins. To identify additional genes involved in innate immunity to HopZ1a, we designed a forward genetics screen based on a loss of HopZ1a recognition. We identified several alleles of the *hopz-effector-triggered-immunity deficient* (*zed1*) mutant. *zed1* is impaired in ZAR1-mediated defense responses but is not affected in the recognition of other unrelated T3SEs or in basal immunity. ZED1 is a previously uncharacterized signaling protein that is modified by HopZ1a. This work reveals novel genes involved in innate immunity, and additional immune signaling pathways in *Arabidopsis*.

P48 The *Pseudomonas syringae* type III effectors HopK1 and AvrRps4 are processed during import into chloroplasts

GUANGYONG LI, JAMES R. ALFANO

Center for Plant Science Innovation and the Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68588, USA

To be pathogenic *Pseudomonas syringae* injects effector proteins into plant cells via its type III secretion system. A primary role for the majority of these effectors is to suppress plant immunity. We focused on one of these effectors, HopK1, because it possessed a robust ability to suppress immunity. A *P. syringae* pv. *tomato* DC3000 *hopK1* mutant was substantially reduced in virulence more so than most single effector mutants, which usually have subtle virulence phenotypes. The N-terminal 147 amino acids of HopK1 share high sequence identity with the well characterized AvrRps4 protein, however, their C-terminal regions are dissimilar. HopK1 is processed inside plant cells at the same site where AvrRps4 has been reported to be processed. Interestingly, transgenic plants expressing HopK1 and AvrRps4 derivatives indicate that both proteins are targeted to chloroplasts using subcellular localization and biochemical fractionation. Additionally, biochemical fractionation experiments using Arabidopsis infiltrated with *P. syringae* strains containing HopK1-HA or AvrRps4-HA indicate that the processed form of these bacterially-injected proteins was found predominately in chloroplasts. Immunity-induced transgenic plants expressing full length HopK1 were reduced in two common immune responses and complemented the virulence phenotype of a DC3000 *hopK1* mutant. However, plants expressing the processed form of HopK1, which would not be localized to chloroplasts, failed to complement the DC3000 *hopK1* mutant and produced immune responses similar to wild type plants suggesting the HopK1 needs to be localized to the chloroplast to function. Taken together, HopK1 and AvrRps4 likely target distinct plant proteins inside chloroplasts to contribute to plant pathogenesis.

P49 The *Pseudomonas* type III effector HopQ1 promotes bacterial growth and interacts with tomato 14-3-3 proteins

WEI LI, G. COAKER

Department of Plant Pathology, University of California, Davis, CA, 95616, USA

Bacterial pathogens deliver multiple effector proteins into host cells to facilitate bacterial growth. HopQ1 is an effector from *Pseudomonas syringae* pv. *tomato* DC3000, which is conserved across many plant bacterial pathogens. Transgenic tomato lines expressing HopQ1 exhibit enhanced susceptibility to bacterial virulence. To identify HopQ1 plant targets, we conducted immunoprecipitations and identified the tomato 14-3-3 proteins TFT1 and TFT5 by mass spectrometry. HopQ1 possesses an N-terminal mode I 14-3-3 binding motif. Four phosphorylation sites were identified in HopQ1's N-terminus by mass spectrometry. Using confocal microscopy, HopQ1, TFT1 and TFT5 fused to GFP localize to the cytoplasm and nucleus in epidermal cells. We are currently investigating the role of HopQ1's 14-3-3 binding site for bacterial virulence and subcellular localization.

P50 Receptor-like cytoplasmic kinases in plant innate immunity

Z. J. D. LIN, J. LIU, G. COAKER

Department of Plant Pathology, University of California at Davis, 210 Hutchison Hall, One Shields Avenue, Davis, California, 95616, USA

Arabidopsis thaliana receptor-like cytoplasmic kinases (RLCKs), are a subset of the receptor like kinases (RLKs) but lack both extracellular and transmembrane domains. Several members of the Arabidopsis RLCK subfamily VII have been conclusively linked to plant innate immunity. The RLCK subfamily VII is rather large, consisting of 46 members, and given the existing lines of evidence it is probable that additional RLCK-VII's may be involved in plant immunity. To identify these additional RLCKs, a meta-analysis of public Arabidopsis microarray experiments was performed to identify RLCKVIIs exhibiting differential regulation in response to biotic stress. T-DNA insertion lines for these particular RLCK-VIIs were obtained and subjected to disease phenotyping. Two were found to exhibit enhanced disease resistance in response to *Pseudomonas syringae* pv. *tomato* DC3000 treatment. Further characterization revealed that these two mutants exhibit altered MPK3/6 activation kinetics. Additional efforts will be undertaken to elucidate the role of these RLCK-VIIs in plant innate immunity. These efforts will entail an examination of well-established plant immune responses in the mutants for aberrant phenotypes and a search for protein interactors using a biochemical approach.

P51 Taking Stalk: Functional analysis of the HopF family of type III secreted effectors from *Pseudomonas syringae*

T. LO, H. O'BRIEN, M. WILTON, B. HURLEY, A. LEE, D. GUTTMAN, D. DESVEAUX

Department of Cell and Systems Biology, Centre for the Analysis of Genome Evolution and Function, University of Toronto, Earth Sciences Building, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada

Armed with a multitude of virulence proteins, termed type III secreted effectors (T3SEs), the phytopathogen *Pseudomonas syringae* is virulent on a broad range of plant hosts. Numerous families of T3SEs exist to promote *P. syringae* infection through suppression of the plant's immune system, including the HopF family. Though research on this T3SE family has focused on two members, the HopF2 from PtoDC3000 and HopF1 from Pph1449B, the importance of other family members have yet to be investigated. This study aims to explore the roles of other HopF family members and to examine the function of the HopF family as a whole. With the increasing number of sequenced *P. syringae* genomes, the HopF family has greatly expanded to over twenty different members. Phylogenetic analysis of this family was performed and studies of the role of positive selection lead to the identification of one positively selected site. The effect of this positively selected site and previously identified catalytic sites were examined on HopF family members. As well, functional characterization of this T3SE family was undertaken on ecotypes of Arabidopsis, common bean and soybean to further elucidate the role of the HopF family in plant–microbe interactions.

P52 HpaP controls type III secretion, pathogenicity and host specificity in R*alstonia solanacearum*

D. LOHOU, M. TURNER, C. PEANNE, A. C. CAZALE-NOEL, S. GENIN, F. VAILLEAU

Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR CNRS-INRA 2594/441, 24 Chemin de Border Rouge, Auzeville, CS 52627, 31326 Castanet Tolosan, France

Ralstonia solanacearum is the causal agent of bacterial wilt on more than 200 plant species including important crops. The bacterium exerts its pathogenicity through more than a hundred secreted proteins, classically called Type 3 Effectors (T3Es), some of them depend directly on the functionality of a Type 3 Secretion System (T3SS). HpaP (*hrp*-associated) is a chaperone-like protein homologous to HpaC in *Xanthomonas campestris* pv. *vesicatoria,* which was shown to interact with T3Es and structural components of the TTSS. We identified HpaP as an important determinant of virulence and host specificity. The *hpaP* mutant of wild type (WT) GMI1000 has reduced pathogenicity on tomato and *Arabidopsis thaliana* and is unable to provoke disease on *Medicago truncatula*. In order to understand what controls the outcome of the interaction with *M. truncatula,* we looked for interactors of HpaP and studied its role in regulating secretion. Yeast two hybrid experiments and pull-down assays revealed interactions between HpaP and a T3E. With experiments on time course of secretion, we observed that the secretion of this HpaP-interacting T3E, as well as a few other T3Es, was modulated by HpaP. Studying the implication of HpaP in the translocation of these T3Es is underway.

P53 Dual Cf-2-mediated disease resistance in tomato requires a common virulence target of a fungus and a nematode

JOSE L. LOZANO-TORRES[1], RUUD H. P. WILBERS[1], PIOTR GAWRONSKI[1], JORDI C. BOSHOVEN[1], ANNA FINKERS-TOMCZAK[1], JAN H. G. CORDEWENER[2], ANTOINE H. P. AMERICA[2], HEIN OVERMARS[1], JOHN W. VAN'T KLOOSTER[3], LUKASZ BARANOWSKI[4], MIROSLAW SOBCZAK[4], MUHAMMAD ILYAS[5], RENIER A. L. VAN DER HOORN[5], ARJEN SCHOTS[1], PIERRE J. G. M. DE WIT[2,6,7], JAAP BAKKER[1,6], ASKA GOVERSE[1,6], GEERT SMANT[1,6]

[1]Laboratory of Nematology and [3]Laboratory of Phytopathology, Wageningen University, 6708 PB Wageningen, The Netherlands; [4]Department of Botany, Warsaw University of Life Sciences, 02-776 Warsaw, Poland; [5]Plant Chemetics Group, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany; [6]Centre for Biosystems Genomics, 6700 AB Wageningen, The Netherlands; [7]King Saud University, Riyadh 11451, Saudi Arabia; [2]Plant Research International, 6708 PB Wageningen, The Netherlands

Plants lack the seemingly unlimited receptor diversity of a somatic adaptive immune system as found in vertebrates and rely on only a relatively small set of innate immune receptors to resist a myriad of pathogens. Here, we show that disease-resistant tomato plants use an efficient mechanism to leverage the limited nonself recognition capacity of their innate immune system. We found that the extracellular plant immune receptor protein Cf-2 of the red currant tomato (*Solanum pimpinellifolium*) has acquired dual resistance specificity by sensing perturbations in a common virulence target of two independently evolved effectors of a fungus and a nematode. The Cf-2 protein, originally identified as a monospecific immune receptor for the leaf mold fungus *Cladosporium fulvum*, also mediates disease resistance to the root parasitic nematode *Globodera rostochiensis* pathotype Ro1-Mierenbos. The Cf-2–mediated dual resistance is triggered by effector-induced perturbations of the apoplastic Rcr3pim protein of *S. pimpinellifolium*. Binding of the venom allergen-like effector protein Gr-VAP1 of *G.rostochiensis* to Rcr3pim perturbs the active site of this papain-like cysteine protease. In the absence of the Cf-2 receptor, Rcr3pim increases the susceptibility of tomato plants to *G. rostochiensis*, thus showing its role as a virulence target of these nematodes. Furthermore, both nematode infection and transient expression of *Gr-VAP1* in tomato plants harboring *Cf-2* and *Rcr3pim* trigger a defense-related programmed cell death in plant cells. Our data demonstrate that monitoring host proteins targeted by multiple pathogens broadens the spectrum of disease resistances mediated by single plant immune receptors.

P54 Complex regulation of the defence-related Arabidopsis transcription factor AtMYB30: from the plant cell to bacterial effectors

DANIEL MARINO, SOLENE FROIDURE, JOANNE CANONNE, ALAIN JAUNEAU, CECILE POUZET, DOMINIQUE ROBY, SUSANA RIVAS

INRA/CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, F-31326 Castanet-Tolosan, France

The MYB transcription factor AtMYB30 was previously identified as a positive regulator of Arabidopsis defense and associated cell death (HR) responses to bacterial pathogens. We recently showed that AtMYB30 is targeted by the *Xanthomonas* type III effector XopD resulting in suppression of AtMYB30-mediated plant defences and underlining the crucial role played by AtMYB30 in the regulation of plant disease resistance. In addition, the activity of AtMYB30 is tightly controlled by plant cells. First, in the presence of AtMYB30, the secreted phospholipase *At*sPLA2-α is partially relocalized to the nucleus where the two proteins interact, leading to repression of AtMYB30-mediated HR and defences. These data highlight the importance of cellular dynamics for defence-associated gene regulation in plants.
Second, new data show that the RING-type E3-ubiquitin ligase protein MIEL1 (AtMYB30-Interacting E3 Ligase1) interacts with AtMYB30 in the plant cell nucleus, leading to AtMYB30 ubiquitination and proteasomal degradation. As a result, MIEL1 negatively regulates AtMYB30-mediated transcriptional activation and Arabidopsis defence and HR responses. Moreover, our results show that MIEL1 negatively regulates plant cell death and defense through AtMYB30 protein degradation under non-pathogenic conditions. After inoculation, repression of *MIEL1* expression removes this negative regulation and allows sufficient AtMYB30 accumulation to trigger HR development.

P55 Possible role of the septum protein ZapA in Citrus / *Xanthomonas citri* subsp. *citri* (Xac) pathosystem

P. M. M. MARTINS, A. M. DO AMARAL, S. C. FARAH, H. FERREIRA

Departamento de Microbiologia Aplicada, UNESP, campus Rio Claro, Brazil

Previous data obtained at our lab demonstrated that GFP-ZapA production into Xac ended up to be detrimental to the full development of its virulence in citrus plants, although no significant difference could be observed in NYG medium growth.
As the mutant strain wasn't knocked-out to the production of ZapA protein, we suspected that the extra production of GFP-ZapA was responsible for this defective phenotype. To asses that, we tested another mutant strain, also producing extra copies of ZapA, and we could see that the excess of this protein *per se* was not the main cause of the virulence loss. We wondered if this could be due to a specific ZapA protein-protein interaction disturbed by the GFP tag in its N terminal domain and a Y2H experiment was carried out to identify this target. The results so far showed no interaction with classical pathogenicity traits, as effectors or any secretion system.
Interestingly, the symptoms appeared to be less severe into the orange host than into the Rangpur Lime, showing that although we still don't have a conclusion of what is causing this loss of virulence, there may be special traits concerning to the host immunity that may cause this variation between them.

P56 SUMO-mediated transcription regulation in stress and innate immune responses

M. J. MAZUR, A. BEINTEMA, V. HAMMOUDI, G. VLACHAKIS, H. A. VAN DEN BURG

Molecular Plant Pathology, SILS, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

Plant defense responses against pathogen invasions are orchestrated by a rapid complex transcriptional reprogramming of host cells, which requires action of both the plant hormones and diverse transcription factors (TFs). Recently, we have shown the emerging role of **s**mall **u**biquitin-**l**ike **m**odifier (SUMO) as a potential regulator of defense–related genes expression in *Arabidopsis.* Our data indicates that in non-infected plants, SUMO conjugation suppresses expression of defense genes. Correspondingly, SUMO null mutants show constitutive SA-dependent defense activation. To date, hundreds of Arabidopsis proteins have been found to either be SUMO1 conjugation targets or interact with the SUMO machinery, including many important plant defense TFs, transcriptional coregulators, chromatin modifying enzymes and heat-shock proteins. To get a comprehensive SUMO protein interaction network in transcriptional reprogramming, we are performing a systematic yeast two-hybrid interaction study, combined with *in vitro* SUMOylation assays. This should reveal SUMO dependent transcription regulation hubs in stress and defense signaling. This network will be used to examine the role of 'non-conserved' plant specific SUMO paralogs in stress and defense responses.

P57 Two potato NAC transcription factors are the targets of a *Phytophthora infestans* effector

H. McLELLAN[1], M. A. ARMSTRONG[1], P. C. BOEVINK[2], P. R. J. BIRCH[1]

[1]Division of Plant Science, College of Life Sciences, University of Dundee (at JHI), Invergowrie, Dundee DD2 5DA, UK; [2]Cell and Molecular Science group, JHI, Invergowrie, Dundee, DD2 5DA, UK

The potato late blight pathogen *Phytophthora infestans* secretes a vast array of effector proteins which are thought to act in its hosts by disarming defences and promoting an environment conducive to pathogen growth and replication. However, very little is known to date about the host targets of these effectors and how they are manipulated by the pathogen. This work describes the identification of two putative membrane bound NAC transcription factors (TF) as the host targets of the RxLR effector Pi03192. The interaction takes place at the ER membrane where these proteins are localised. Silencing of *NAC1* and *2* in *Nicotiana benthamiana* is shown to compromise resistance to *P. infestans* establishing the role of these genes in defence. Transcription of *NAC1* and *2* is rapidly induced by *Phytophthora* PAMP treatment and StNAC1 and 2 proteins are released from the endoplasmic reticulum (ER) membrane on PAMP treatment and are rapidly turned over in the nucleus by the proteasome. The virulence mechanism of Pi03192 is to block the translocation of StNAC1 & 2 into the nucleus, this is a novel mode of action for a plant pathogen effector.

P58 Effectoromics

RICHARD MICHELMORE[1], JEAN GREENBERG[2], JOAN WONG[1], JULIANA GIL[1], TADEUSZ WROBLEWSKI[1], BERTRAND PERROUD[1], JIYOUNG LEE[2], KERI CAVANAUGH[1], YONGSUNG KANG[2], JOANNA JELENSKA[2], LUTZ FROENICKE[1], LIDA DEREVNINA[1]

[1]The Genome Center, University of California, Davis, CA 95616, USA; [2]Department of Molecular Genetics & Cell Biology, The University of Chicago, Chicago, IL 60637, USA

We have several projects aimed at effector discovery and functional analysis. For effector discovery, we are sequencing several downy mildews to enable comparative genomics across the Peronosporaceae. We are sequencing the genome of *Bremia lactucae* to characterize the effector repertoire of the most important pathogen of lettuce. This has been challenging due its high level of heterozygosity. A series of hybrid assemblies have been made using sequences from several technologies. Candidate effector sequences have been identified using the LxLR and EER motifs. We are also sequencing several other downy mildews, including *Sclerospora graminicola* and *Peronosclerospora sorghi* to provide sequences of effectors from these important tropical downy mildews. Functional analysis of effectors from diverse pathogens has been made using *Agrobacterium*- and TRV-mediated transient *in planta* expression and yeast two-hybrid analysis of protein-protein interactions. Expression of *B. lactucae* effectors in a differential series of resistant lettuce cultivars failed to detect any that elicited a necrotic reaction, reflecting the adaptation of *B. lactucae* to its host, similar that observed with bacterial pathogens. Yeast two hybrid analysis has been made with three libraries: pathogen effectors, plant NBS-LRR proteins, and plant signaling proteins. Numerous interactions have been identified consistent with the idea that effectors from diverse pathogens target common points of vulnerability within the plant.

P59 Suppression of apoplastic immunity by *Ustilago maydis*

A. MÜLLER[1], K. VAN DER LINDE[1], C. HEMETSBERGER[1], C. HERRBERGER[1], S. ZIEMANN[1], R. VAN DER HOORN[2], G. DOEHLEMANN[1]

[1]Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Str. 10, D-35043 Marburg, Germany; [2]Plant Chemetics Lab, Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, D-50829 Cologne, Germany

The smut fungus *Ustilago maydis* causes the formation plant tumors on all aerial organs of its host plant maize. A compatible, biotrophic interaction is established directly upon host penetration and is maintained during the entire interaction. This biotrophic lifestyle requires an efficient suppression of plant defense responses. Effector proteins, which are secreted by biotrophic hyphae, are considered to be instrumental for the modulation of host immunity.
However, only little is known about the molecular function of the individual effector proteins.
Our recent findings suggest that conserved components of the apoplastic plant defense machinery are direct targets of *U. maydis* effectors. This particularly involves enzymes such as peroxidases and cysteine proteases, which are crucial for the activation of salicylic-acid dependent defense reactions in maize. We now show that a set of cysteine proteases is inhibited both by a *U. maydis*-induced maize cystatin as well as by the secreted effector Pit2, and both these factors are essential for compatibility. Molecular analysis of Pit2 also identified a conserved sequence motif, which mediates the inhibition of plant cysteine proteases and is therefore essential for fungal virulence. Together, the presented data provide new insights on the suppression of apoplastic immunity by a biotrophic fungal pathogen.

P60 Functional analysis of *Ustilago maydis* effector Tin3 of cluster 19A

NINA NEIDIG, THOMAS BREFORT, REGINE KAHMANN

Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany

Ustilago maydis is a biotrophic fungal plant pathogen that causes smut disease in its host plant maize. Previous genomic studies revealed that *Ustilago maydis* depends on a variety of novel secreted effector proteins to establish a compatible interaction with its host plant. With respect to tumor formation cluster 19A encoding 23 effectors is of special interest, as cluster mutants still proliferate inside the plant tissue but fail to produce tumors. Especially *tin3* (*tumor inducing 3*), a unique gene, contributes significantly to tumor formation. Using fluorescence microscopy and immunoblot-analysis it was shown that Tin3 is secreted into the apoplastic space and that it accumulates at hyphal tips and cell-to-cell passages. In Yeast-two-hybrid approaches two interesting interaction partners for Tin3 were identified: Mir3, a plant defense related cysteine protease and Beclin1, an autophagy related protein of maize. We present evidence that Tin3 is inhibiting the protease activity of Mir3 and related proteases in in-vitro proteaseassays and in plant lysates of infected maize plants. Complementation studies show the biological relevance of this function for pathogenicity. In addition, we are currently investigating the influence of Tin3 on autophagy in infected host-tissue using a variety of microscopic techniques. With these studies we aim to assess the range of functions of Tin3 during biotrophic development of *U. maydis*.

P61 Functional analysis of candidate effectors in the poplar leaf rust fungus *Melampsora larici-populina*

BENJAMIN PETRE[1], HUGO GERMAIN[2], DAVID L. JOLY[2], STÉPHANE HACQUARD[1], ARNAUD HECKER[1], NICOLAS ROUHIER[1], ARMAND SÉGUIN[2], SÉBASTIEN DUPLESSIS[1]

[1]UMR 1136 Interactions Arbres/Microorganismes, INRA/Université de Lorraine, Centre INRA de Nancy, Champenoux, France; [2]Canadian Forest Service, Laurentian Forestry Centre, Québec, Canada

Poplars are extensively cultivated worldwide for wood production but their susceptibility to the leaf rust fungus *Melampsora larici-populina* leads to considerable damages in plantations. *Populus trichocarpa* and *M. larici-populina* were the first tree and rust fungus genomes sequenced, making this pathosystem a model for post-genomic studies in forest pathology. Recent works established catalogues of small secreted proteins (SSP) considered as candidate effectors likely required for the biotrophic growth of the rust fungus. In particular, two gene families encode modular SSP with a conserved N-terminal part and a C-terminal part evolving under positive selection except highly-conserved cysteine residues. Gene family 5464 contains 13 members, homologs of *Melampsora lini* AvrP4 avirulence factors whereas H1 family gather 31 genes specific to *M. larici-populina*. Some of these genes present a transient peak of expression during the biotrophic growth of the fungus in poplar leaves, and the corresponding proteins are able to enhance bacterial growth when delivered in *Arabidopsis thaliana* from *Pseudomonas syringae* pv *tomato* DC3000. Yeast-double hybrid and pull-down assays were used in a screen to identify interacting proteins in Arabidopsis and poplar. To go further into effectors characterisation, we are investigating biochemical and structural properties of corresponding recombinant proteins.

P62 Comprehensive Analysis of *Piriformospora indica* candidate effector proteins

MARYAM RAFIQI[1], LOCHNIT GÜNTER[2], JELONEK LUKAS[1], ZHANG FENG[1], DAGMAR BIEDENKOPF[1], KARL-HEINZ KOGEL[1]

[1]Institute of Phytopathology and Applied Zoology, Research Centre for BioSystems, LandUse and Nutrition (IFZ), Justus Liebig University, Giessen, Germany; [2]Institute of Biochemistry, Justus Liebig University, Giessen, Germany

One of the most exciting developments in plant-microbe interactions has been the finding that both pathogenic and mutualistic fungi deliver effector proteins into the cytoplasm of host cells. Whole genome sequencing of *P. indica* has thrown up many sequences encoding for small secreted proteins (SSPs) that could function as effectors. Presently, the selection of fungal SSPs relies largely on transcriptomic data. SSPs are known to be upregulated during *in planta* growth. However, information about the presence and abundance of proteins encoded by these SSP genes is usually lacking. Here, we develop a high throughput comprehensive approach to further characterise *P. indica* list of effector candidates. We used *in silico* analysis, using a set of prediction algorithms (SignalP, TargetP, TMHMM, MCL, T-reks), combined with proteomic screening, (using 2D gel, mass spectrometry and peptide mass mapping) to identify effector protein candidates abundant in colonised plant tissue at different stages of biotrophic fungal growth.

P63 Conservation of RIN4 function in Arabidopsis and soybean

THOMAS REDDITT, TOM ASHFIELD, ANDREW RUSSELL, NATALIE RODIBAUGH, ROGER INNES

Department of Biology, Indiana University - Bloomington, Myers Hall 150, 915 East Third Street, Bloomington, IN 47405, USA

Arabidopsis and soybean are both capable of detecting the bacterial effectors, AvrB and AvrRpm1. We are investigating whether these species use similar mechanisms to recognize AvrB and AvrRpm1, even though the *R*-genes responsible for this recognition are not orthologous and have evolved independently of one another. To test these pathways, we attempted to complement mutant Arabidopsis that lacked *rpm1, rin4*, or both with the appropriate soybean genes. Arabidopsis *rps2 rin4* mutants were transformed with each of the four co-orthologous soybean *RIN4* genes (*GmRin4A/B/C/D*.) While, Arabidopsis *rpm1 rps2 rin4* mutants were transformed with *Rpg1-b,* one *GmRin4*, or both. Preliminary results show that *GmRin4C* is able to complement the Arabidopsis *rin4* mutation in RPM1-mediated defense against both AvrB and AvrRpm1, while *GmRin4D* is only able to complement for recognition of AvrB. Both *GmRin4A* and *GmRin4B* were unable to complement the *rin4* mutation for recognition of either bacterial effector, suggesting a functional difference between the GmRin4s. However, preliminary data suggest that *Rpg1-b* is unable to complement for AvrB recognition in *rpm1* mutant plants, even in the presence of any *GmRin4*. Future directions will focus on identifying the biochemical activities of AvrB and AvrRpm1 that lead to detection in both Arabidopsis and soybean.

P64 Eicosapolyenoic acids as novel PAMPs with reciprocal effects on plant defense signaling networks

S. M. ROBERTS[1], M. L. BJORNSON[2], T. SAVCHENKO[2], T. V. ROUBTSOVA[1], M. F. PYE[1], T. KASUGA[3], C. LAZARUS[4], K. DEHESH[2], R. M. BOSTOCK[1]

Departments of [1]Plant Pathology and of [2]Plant Biology, University of California, Davis, CA 95616, USA; [3]USDA-ARS, Davis, CA, USA; [4]School of Biological Sciences, University of Bristol, Bristol, UK

Eicosapolyenoic acids (EP) – arachidonic (AA) and eicosapentaenoic (EPA) acids – are common fatty acids in lipids and cellular components of plant pathogenic oomycetes that function as conserved signaling molecules across eukaryotic kingdoms. EP released during infection of plants by *Phytophthora* species may serve as novel PAMPs to engage defense signaling networks in a jasmonic acid-dependent manner. These changes are manifested as a generalized rapid stress response resulting in enhanced tolerance to pathogens and insects. Transcriptome analyses of *Arabidopsis* roots challenged with *Phytophthora capsici* or engineered to constitutively produce very low levels of EPs reveal a coexpression network of four strongly induced genes. The AA-induced expression pattern and known functions of these genes implicates them in a stress response network in *Arabidopsis* that serves some unique function in AA response. AA and EPA action in *Arabidopsis* and in a tomato root model will be presented.

P65 Towards understanding the role of aphid effectors in promoting susceptibility

PATRICIA RODRIGUEZ, ANNIKA NITSCHE, TIM WARBROEK, JORUNN BOS

Cell and Molecular Sciences, James Hutton Institute, Invergowrie, DD2 5DA, UK

Like most plant parasites, aphids require intimate associations with their hosts to gain access to nutrients. Aphids predominantly feed from the phloem, and have stylets that navigate through different layers of leaf tissue to form an interface with the host where signals are exchanged. Indeed, aphid feeding induces clogging of phloem sieve elements, which is suppressed by aphids in successful interactions. In addition, some aphid species alter host phenotypes by inducing gall formation or leaf curling. Suppression of host defences and altering plant physiology is common among plant pathogens and involves secretion of effectors. Recent evidence suggests that aphids, like other plant parasites, secrete effectors that share functional features with plant pathogen effectors. The identification and characterization of such effectors is crucial to gain insight into the molecular mechanisms underlying resistance and susceptibility to aphids. Our research aims to identify aphid effectors and their host targets to understand their involvement in host cell manipulation. As part of our efforts, we screened candidate effectors from the aphid species *Myzus persicae* using yeast-two-hybrid assays. Here, we will report our progress on identifying and characterizing aphid effector-target interactions.

P66 Large-scale gene disruption in *Magnaporthe oryzae* identifies MC69, a secreted protein required for infection by monocot and dicot fungal pathogens

HIROMASA SAITOH[1], CHIKAKO MITSUOKA[1], AKIKO HIRABUCHI[1], KYOKO IKEDA[2], HIROKI IRIEDA[2], KAE YOSHINO[2], KENTARO YOSHIDA[3], JOE WIN[3], SOPHIEN KAMOUN[3], YOSHITAKA TAKANO[2], RYOHEI TERAUCHI[1]

[1]Iwate Biotechnology Research Center, Narita 22-174-4, Kitakami, Iwate 024-0003, Japan; [2]Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-Cho, Sakyo-ku, Kyoto 611-0011, Japan; [3]The Sainsbury Laboratory, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

To search for virulence effector genes of the rice blast fungus, *Magnaporthe oryzae*, we carried out a large-scale targeted disruption of genes for 78 putative secreted proteins that are expressed during the early stages of infection of *M. oryzae*. Disruption of the majority of genes did not affect growth, conidiation, or pathogenicity of *M. oryzae*. One exception was the gene *MC69*. The *mc69* mutant showed a severe reduction in blast symptoms on rice and barley. The *mc69* mutant did not exhibit changes in saprophytic growth and conidiation. Microscopic analysis of infection behavior in the *mc69* mutant revealed that MC69 is dispensable for appressorium formation. However, *mc69* mutant failed to develop invasive hyphae after appressorium formation in rice leaf sheath. *MC69* encodes a hypothetical 54 amino acids protein with a signal peptide. Live-cell imaging suggested that fluorescently labeled MC69 was not translocated into rice cytoplasm. Furthermore, deletion of the *MC69* orthologous gene reduced pathogenicity of the cucumber anthracnose fungus *Colletotrichum orbiculare* on both cucumber and *Nicotiana benthamiana* leaves. We conclude that MC69 is a secreted pathogenicity protein commonly required for infection of two different plant pathogenic fungi, *M. oryza*e and *C. orbiculare* pathogenic on monocot and dicot plants, respectively.

P67 Rhamnolipids elicit plant defence responses and enhance resistance against biotrophic, hemibiotrophic and necrotrophic phytopathogens

L. SANCHEZ, B. COURTEAUX, C. CLEMENT, F. BAILLIEUL, S. DOREY

URVVC-EA 4707, Stress, Défenses et Reproduction des Plantes, Université de Reims Champagne-Ardenne, BP 1039, F-51687 Reims cedex 2, France

Rhamnolipids (RLs) are amphiphilic secondary metabolites produced by bacteria. Because they are potentially at the plant cells interface, we investigated their role as Microbe-Associated Molecular Pattern. In grapevine, RLs stimulate Ca^{2+} influx, mitogen-activated protein kinase activation and reactive oxygen species production. RLs also induce the expression of several defence genes. RLs efficiently protect grapevine against *Botrytis cinerea*. In *Arabidopsis thaliana* RLs trigger an immune response that participates to the plant resistance against the hemibiotrophic bacterium *Pseudomonas syringae* pv. *tomato*, the biotrophic oomycete *Hyaloperonospora arabidopsidis* and necrotrophic fungus *Botrytis cinerea*. RL-mediated resistance involves different signalling pathways that depend on the type of pathogen. ET is involved in RL-induced resistance to *H. arabidopsidis* and to *P. syringae* whereas JA is essential for the resistance to *B. cinerea*. SA participates to the restriction of all pathogens. SA dependent plant defences are potentiated by RLs following challenge by *B. cinerea* or *P. syringae*. These results highlight a central role for SA in RL-mediated resistance. In addition to the activation of plant defence responses, antimicrobial properties of RLs participate in the protection against the fungus and the oomycete. RLs and lipopeptides can be considered as a new class of amphiphilic molecules perceived by plant cells and particularly efficient to protect plant against pathogens.

P68 Host protein BSL1 associates with *Phytophthora infestans* RXLR effector PiAVR2 and the immune receptor R2 to mediate disease resistance

DIANE G.O. SAUNDERS[1], SUSAN BREEN[2,3], JOE WIN[1], SEBASTIAN SCHORNACK[1], INGO HEIN[2], TOLGA O. BOZKURT[1], NICOLAS CHAMPOURET[1], VIVIANNE G.A.A. VLEESHOUWERS[4], PAUL R.J. BIRCH[2,3], ELEANOR M. GILROY[2], SOPHIEN KAMOUN[1]

[1]The Sainsbury Laboratory, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK; [2]Cell and Molecular Sciences, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK; [3]Division of Plant Sciences, University of Dundee (at JHI), Invergowrie, Dundee, DD2 5DA, UK; [4]Wageningen UR Plant Breeding, 6700 AJ, Wageningen, The Netherlands

Plant pathogens secrete effectors to modulate plant immunity and promote host colonization. Little is known about how plant nucleotide-binding leucine-rich repeat (NB-LRR) immunoreceptors recognize effectors of filamentous plant pathogens such as *Phytophthora infestans*. PiAVR2 belongs to a family of thirteen *P. infestans* RXLR effectors differentially recognized by members of the R2 NB-LRR family in potato. We used *in planta* coimmunoprecipitation to identify host targets of PiAVR2. We discovered that PiAVR2 associated *in planta* with BSL1, a putative phosphatase. Furthermore, the five PiAVR2 family members that are recognized by R2 associated with BSL1 *in planta*, suggesting a role for BSL1 in R2-mediated recognition. We also discovered that PiAVR2 mediates an interaction between BSL1 and R2, possibly through the formation of a ternary complex. Strains of *P. infestans* that are virulent on R2 potatoes express an unrecognized form *PiAvr2-like* (*PiA2l*). PiA2L still interacted with BSL1 but did not promote the association of BSL1 with R2. Our findings show that recognition of the *P. infestans* PiAVR2 effector by R2 requires BSL1. This reveals that, similar to effectors of phytopathogenic bacteria, recognition of filamentous pathogen effectors can be mediated via a host protein that interacts with both the effector and the NB-LRR immunoreceptor.

P69 Utilization of *Burkholderia glumae* type III secretion system for translocating pathogen effectors to monocot cells

SHAILENDRA SHARMA[1], SHIVETA SHARMA[1], KENTARO YOSHIDA[1,2], KOKI FUJISAKI[1], AKIKO ITO[1], RYOHEI TERAUCHI[1], SOPHIEN KAMOUN[2], KEE HOON SOHN[2], JONATHAN D.G. JONES[2], HIROMASA SAITOH[1]

[1]Iwate Biotechnology Research Center, Kitakami, Iwate 024-0003, Japan; [2]The Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, UK

Many phytopathogenic gram negative bacteria employ type III secretion system (T3SS) to inject the effector proteins from their cytosol to eukaryotic host cells. Identification and characterization of novel effector proteins is important to understand pathogen virulence and host plant defense capabilities. Genome sequencing information of many plant fungal pathogens have enabled searches for novel effectors. In the present study, we report a pEDVbased effector delivery system in which T3SS of *Burkholderia glumae*, an emerging rice pathogen, is used to translocate the AVR-Pik and AVR-Pii effectors of blast pathogen *Magnaporthe oryzae* to rice cytoplasm. Translocated AVR-Pik and AVR-Pii showed avirulence activity for incompatible interaction in rice cultivars containing cognate R genes. AVR-Pik showed a reduced and delayed hypersensitive response in non-host plant *Nicotiana bethamaina* suggesting its virulence activity. After delivery by *B. glumae* T3SS, the AVRs fused with fluorescent protein and nuclear localization signal (NLS) were observed in the nuclei of cells of rice, wheat, barley and *N. bethamaina*. Effector delivery and localization assay, described in the present study, provides an efficient method to identify and study the function of effector proteins in the monocots.

P70 Protein microarray platform for pathogen effectors studies and beyond

SHISONG MA[1,2], SMIT SHAH[1,2], JONGCHAN WOO[1,2], KERI CAVANAUGH[2], RICHARD MICHELMORE[2], JOHN MCDOWELL[3], BRETT TYLER[4], MICHAEL SNYDER[5], S. P. DINESH-KUMAR[1,2]

[1]Dept. of Plant Biology and [2]The Genome Center, UC Davis, CA 95616, USA; [3]PPWS Department, Virginia Tech, Blacksburg, VA 24061, USA; [4]Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA; [5]Department of Genetics, Stanford University, Stanford, CA 94305, USA

Protein microarray technology has emerged as a powerful approach for the study of hundreds or thousands of proteins simultaneously. Since the majority of pathogen effector proteins lack discernable enzymatic or biochemical functions, large-scale characterization of these proteins will depend on the identification of their host targets. Therefore, we are developing plant protein microarrays and pathogen effector microarrays for the comprehensive biochemical and functional analysis of pathogen effectors. We will discuss our efforts towards application of these protein microarrays to understand effector biology.

P71 The role of secreted LysM domain proteins during development of *Ustilago maydis*

NANCY STOLLE, KARIN MÜNCH, REGINE KAHMANN

Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany

The biotrophic maize pathogen *Ustilago maydis* relies on the secretion of effector proteins to establish a compatible interaction. In this context, two putatively secreted LysM-containing proteins (*um11464*, *um05087*) have been identified. LysM effectors are very common in pathogenic fungi and have been shown to contribute to virulence.
We have generated deletion mutants of *um11464* and *um05087*. While deletion of *um05087* did not show any phenotype, *um11464* deletion mutants displayed bipolar growth and formed significantly more lateral buds in axenic culture compared to the progenitor strain. Plant infections with *um11464* deletion mutants lead to hypervirulence whereas overexpression of the gene *um11464* reduced tumor formation. Hypervirulence is likely to result from an increased number of appressoria-forming filaments seen in *um11464* mutant strains.
Electron microscopy revealed that the cell wall of *um11464* deletion mutants is thinner and less dense compared to the progenitor strain. Furthermore the mutant lacks fimbriae on its outer cell surface layer and shows higher sensitivity to cell wall stressors. Based on this we consider it likely that Um11464 might have a structural function and might contribute to cell wall integrity. *In situ* immunolocalization studies revealed that Um11464 is located predominantly at the cell periphery. Interestingly, the cell wall attachment is achieved via the C-terminus of Um11464 but is independent of the LysM domains. Currently, functional analyses are performed to investigate the mechanisms of cell wall alterations and how Um11464 affects virulence negatively.

P72 *Xanthomonas* T3S effector XopX suppresses effector triggered immunity to promote pathogenesis

WILLIAM STORK, MICA SORIANO, JUNG-GUN KIM, MARY BETH MUDGETT

Biology Department, Stanford University, Gilbert Hall 224, Stanford, CA, USA

The *Xanthomonas campestris vesicatoria* (Xcv) T3E XopX is an Xcv virulence factor that appears to suppress host defense, but details of XopX activity and potential host targets are unknown. XopX is a 699-amino acid protein conserved among most known *Xanthomonas* strains. Highly conserved motifs among XopX variants include an N-terminal alanine rich region and a predicted tyrosine phosphorylation site at amino acid 275. XopX alleles are also homologous to the *Xanthomonas* T3E Early Chlorosis Factor (ECF). The closest XopX homolog in *Pseudomonas syringae* is HopAE1. Here we demonstrated that XopX suppresses the hypersensitive response (HR) elicited by *Xcv* in the non-host *Nicotiana tabacum*. We performed an *Agrobacterium* mediated transient expression screen of an *Xcv* T3E library to identify T3Es eliciting HR in *N. tabacum*. Finally, we demonstrated that an *Xcv ΔxopX* strain elicits immunity and has reduced growth in a resistant line of tomato, the natural Xcv host species. Future work will determine whether XopX activity is specific to effector triggered immunity. We seek to biochemically characterize XopX virulence function and host targets by performing a structure-function analysis of XopX to identify domains and residues required for its activity and identifying host proteins that interact with XopX through protein-protein interaction analyses.

P73 Enhanced biosynthesis of defense enzymes and resistance to *Ralstonia solanacearum* are mediated through rhizosphere plant growth promoting fungus in tomato

J. SUDISHA, SHIN-ICHI ITO

Laboratory of Molecular Plant Pathology, Department of Biological & Environmental Sciences, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi, Yamaguchi 753-8515, Japan

Beneficial soil-borne microorganisms can induce an enhanced defensive capacity in above-ground plant parts that provides protection against a broad spectrum of microbial pathogens and even insect herbivores. Seventy nine plant growth promoting fungus (PGPF) were isolated from rhizosphere soil of healthy food crops and grass plants of India. Among the tested PGPF isolates, nine isolates showed saprophytic ability, root colonization, phosphate solubilization, IAA production and plant growth promotion. Under laboratory, five PGPFs upon seed priming followed by root dip treatment before transplantation to highly susceptible tomato cultivar Fukuju-2 showed early seedling emergence and enhanced vigor compared to untreated control. Under greenhouse conditions, four isolates significantly increased root length, shoot length, fresh weight, dry weight, fruit yield and total NPK content of 45-dayold-seedlings with respect to control. Maximum disease protection of 57% was noticed in *Trichoderma harzianum* treated plants followed by *Penicillium crysogenum* (45%). PGPF pretreated tomato seedlings, which were later inoculated with *R. solanacearum* showed enhanced activities of PAL, POX, β-1,3 glucanase and LOX. Further expression studies of the defense genes triggering plant inmate to resistance using microarray and validation by qRT-PCR is in progress. These results indicate that PGPFs treatments are an added advantage for advantageous cultivation of tomato.

P74 Structural basis for interactions of the *Phytophthora sojae* RXLR effector Avh5 with phosphatidylinositol 3-phosphate and for host cell entry

FURONG SUN[1,2], SHIV KALE[3], HUGO AZURMENDI[2], DAN LI[1], BRETT M. TYLER[3,4], DANIEL CAPELLUTO[1]

[1]Dept of Biological Sciences, [2]Dept of Chemistry, [3]Virginia Bioinformatics Institute, Virginia Tech, USA; [4]Center for Genome Research and Biocomputing, Oregon State University, USA

Oomycetes, such as *Phytophthora sojae*, employ protein effectors that enter host cells to facilitate infection. Entry of some effector proteins into plant cells is mediated by conserved RxLR motifs in the effectors and phosphatidylinositol 3-phosphate (PI3P) in the host plasma membrane. We have structurally and functionally characterized the *P. sojae* effector Avh5 and its interactions with PI3P. Using NMR spectroscopy, we demonstrate that Avh5 is helical in nature with a long N-terminal disordered region, like related effectors such as *P. infestans* Avr3a. Heteronuclear single quantum coherence titrations of Avh5 with the PI3P head group, inositol 1,3-bisphosphate (IP2), allowed us to identify a C terminal lysine-rich helical region (helix 2) as the principal lipid-binding site in the protein, with the N terminal RxLR (RFLR) motif playing a more minor role. Mutations in the RFLR motif reduced PI3P binding, while mutations in the basic helix almost abolished it. Mutations in the RFLR motif or in the basic region of Avh5 both significantly reduced protein entry into plant and human cells. Both regions independently mediated cell entry via a PI3P-dependent mechanism. Our findings support a model in which Avh5 initially interacts with PI3P through its C-terminal region, enabling the RFLR domain to promote PI3P-mediated host entry.

P75 Search for Arabidopsis components targeted by PopP2 acetyltransferase activity

C. TASSET, G. HUET, A. JAUNEAU, C. POUZET, Y. MARCO, L. DESLANDES

INRA, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, F-31326 Castanet-Tolosan, France; CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR2594, F-31326 Castanet-Tolosan, France

Type III effector proteins from bacterial pathogens manipulate host components to suppress defence responses and promote pathogen development. The *Ralstonia solanacearum* effector protein PopP2 triggers immunity in Arabidopsis following its perception by RRS1-R, its cognate resistance protein. PopP2, that belongs to the YopJ-like family of cysteine proteases, displays acetyltransferase activity and autoacetylates on a particular lysine residue which is well conserved among all members of the YopJ family. Mutation of this lysine residue in PopP2 prevents PopP2 autoacetylation and correlates with its loss of avirulence activity. Activation of RRS1-R-triggered immunity may be conditioned by perception of PopP2 autoacetylation and/or acetylation of host components targeted by PopP2 activity (called PATs for PopP2 acetylated targets). In order to identify such components, the screening of a Y2H Arabidopsis cDNA library was performed using PopP2 as a bait. Among various prey candidates, we identified PAT1, a protein of unknown function that is targeted to the plant nucleus where it physically associates with PopP2. Immunoprecipitation assays reveal that PAT1 is acetylated by PopP2. The functional characterization of PAT1 is in progress and should significantly contribute to the understanding of the molecular role(s) played by protein acetylation during plant innate immunity.

P76 A TTM superfamily member, *AtCYDP2*, displays enhanced resistance to oomycetes and bacteria.

H. UNG, W. MOEDER, K. YOSHIOKA

Department of Cell & Systems Biology, University of Toronto, 25 Willcocks Street, Toronto ON, M5S 3B2, Canada

The members of the triphosphate tunnel metalloenzyme (TTM) superfamily are a group of enzymes that can hydrolyze a variety of polyphosphate substrates. They perform divergent functions in different organisms, such as RNA capping, thiamine metabolism, and cAMP formation. TTM members exist in all domains of life; however, no information is available about the function of any members in plants. In *Arabidopsis*, 3 genes are annotated as TTM family members (*AtCYDP1*, *2*, and *3*). Pathogen infection studies using *AtCYDP2* knockouts (KOs) showed an enhanced resistance phenotype to strains of *Hyaloperonospora arabidopsidis* and *Pseudomonas syringae* that are recognized by TIR class R proteins. They also show enhanced hypersensitive response (HR), a form of programmed cell death at the site of infection. Epistatic analyses were performed to understand the *AtCYDP2*-mediated signal cascade, revealing its dependence on *phytoalexin-deficient4* (*PAD4*), *non-expresser of pathogenesis-related genes1* (*NPR1*), and salicylic acid. Interestingly, preliminary enzymatic analysis revealed phosphatase activity for AtCYDP2, suggesting a role for triphosphate catalysis in mediating HR in plant immunity.

P77 Two MiSSPs (Mycorrhiza induced Small Secreted Proteins) from the mutualistic fungal symbiont *Laccaria bicolor* are required for symbiosis development.

C. VENEAULT-FOURREY[1], Y. DAGUERRE[1], J. PLETT[1], A. KOHLER[1], M. KEMPPAINEN[2], A. PARDO[2], A. BRUN-JACOB[1], F. MARTIN[1]

[1]UMR1136 IaM, Ecogenomics Lab, INRA-Lorraine Université, 54280 Champenoux, France; [2]Micología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes and CONICET. Roque Sáenz Peña 352, B1876 Bernal, Provincia de Buenos Aires, Argentina

Boreal and temperate forest ecosystems rely on ectomycorrhizal (ECM) symbiosis for trees nutrition, productivity and stress resilience. ECM appears several times during fungi evolution and ECM fungi likely derived from saprotrophic ancestors. Despite their ecological importance, very little is known on the molecular dialogue that occurs between tree roots and ECM fungi to sustain the development of symbiosis. Recently, *L. bicolor* Mycorrhizal induced Small Secreted Protein7 (MiSSP7) has been proved to be the first symbiotic effector required for the development of symbiosis due to its targeting to the plant nucleus (Plett *et al*., 2011). Transcriptomic analyses reveal the presence of several additional MiSSPs of unknown function in the genome of the symbiotic fungus *L. bicolor* (Martin *et al*., 2008). We have performed functional analysis of several MiSSPs in order to (i) demonstrate these MiSSPs are required for symbiosis development and (ii) to identify which plant compartment / proteins are targeted by MiSSPs. We will present and discuss our last results with regards to the putative role of MiSSPs as fungal effectors. Plett et al., 2011, *Current Biology*, 21(14):1197−1203; Martin et al., 2008 *Nature* 452, 88−92.

P78 The cyst nematode 30D08 effector targets host nuclear functions

A. VERMA, C. LEE, F. ODU, S. MORRISS, C. KENNING, T. HEWEZI, E. L. DAVIS, T. J. BAUM, R. HUSSEY, M. G. MITCHUM

Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA

Cyst nematodes use a hollow mouth spear or stylet to deliver effector proteins into host cells to establish a metabolically hyperactive feeding site called a syncytium. Several dozen candidate and confirmed sytlet-secreted effectors (SSEs) have been identified from the soybean cyst nematode *Heterodera glycines.* Among these, 30D08 is a novel candidate SSE predicted to localize to host cell nuclei. To facilitate functional studies in Arabidopsis, a homologous *30D08* gene was cloned from *Heterodera schachtii*, a parasite on Arabidopsis. A series of assays including RNA-mediated gene silencing, *in planta* overexpression, localization, and interaction studies are underway to help elucidate the role of 30D08. Transient expression assays in onion epidermal cells confirmed 30D08 localization in plant nuclei. A yeast two-hybrid screen to identify potential host targets identified specific interaction of 30D08 with a nuclear-targeted Arabidopsis protein involved in pre-mRNA splicing. Bimolecular fluorescence complementation and co–immunoprecipation methods are being used to verify the interaction *in planta*. Expression of the targeted host protein in feeding sites was confirmed and knock-out mutants are being studied for any impairment of nematode parasitic function. Our studies suggest that 30D08 is targeted to the plant nucleus where it interacts with host proteins to manipulate nuclear functions of host cells to promote parasitism.

P79 A screen for *H. glycines* stylet-secreted effectors that can suppress programmed cell death in yeast and plants

J. WANG, L. WASALA, G. YECKEL, T.J. BAUM, E.L. DAVIS, R.S. HUSSEY, M.G. MITCHUM

Division of Plant Sciences, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA

Plant pathogens deliver diverse effector proteins into host cells to suppress plant defense and promote infection. While numerous effectors that suppress plant defense have been identified from bacteria, fungi, and oomycete pathogens, relatively little is known for nematode effectors. Nematode effector proteins are injected into host cells using a hollow mouth spear or stylet. Successful parasitism appears to require multiple stylet-secreted effectors (SSEs). Several dozen candidate and confirmed SSEs have been reported from the cyst nematode *Heterodera glycines*. Here, forty-six of these SSEs were screened for a potential role in suppression of programmed cell death (PCD) in yeast. From this screen, we have identified several SSEs that can interfere with PCD. Additional bioassays are underway to determine if these SSEs may function to suppress the HR and other hallmarks of plant defense. The identification of a set of suppressors in *H. glycines* should facilitate ongoing investigations of the underlying functions of SSEs in nematode pathogenesis.

P80 Characterizing interactors of *Hpa* effector HaRxL106 reveals a novel regulatory role for RCD1 in NPR1-mediated immunity

L. WIRTHMUELLER, S. ASAI, G. FABRO, G. RALLAPALLI, M.-C. CAILLAUD, M. J. BANFIELD, J. D. JONES

John Innes Centre/The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

The HaRxL106 effector protein from the oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) suppresses basal defense gene expression when constitutively expressed in *Arabidopsis thaliana*. HaRxL106 also suppresses autoimmunity triggered by the constitutively active TIR-NB-LRR protein variant snc1-1 but has no effect on resistance mediated by two other TIR-NB-LRR proteins, RPP2 and RPP4. The HaRxL106 effector protein localizes exclusively to the nucleus and interacts with several nuclear host proteins, including alpha-importins and RADICAL INDUCED CELL DEATH1 (RCD1). Whereas alpha-importin single knock outs only weakly compromise resistance to virulent *Hpa* races, *rcd1* mutants exhibit loss of basal resistance comparable to an *npr1* mutant. A comparative transcriptomics approach revealed a partially overlapping gene set mis-regulated in transgenic lines expressing HaRxL106 and in *rcd1* mutants. Particularly, NPR1 target genes were underexpressed in both genetic backgrounds before and 24h after infection with virulent *Pseudomonas syringae* DC3000. As *NPR1* transcript levels were not affected by HaRxL106 or *rcd1* our data suggest that RCD1 is required for NPR1 protein function. We will report on our analysis of transgenic lines expressing NPR1-GFP in an *rcd1* mutant background. We hypothesize that HaRxL106 may target RCD1 to suppress NPR1-mediated local and systemic defense.

P81 Effectoromics approach in dissecting the interaction between *P. syringae* pv. phaseolicola and its non-host plant *A. thaliana*

TADEUSZ WROBLEWSKI, KERI CAVANAUGH, NATALIA BELTER, RICHARD W. MICHELMORE

The Genome Center, University of California in Davis, One Shields Ave., Davis, CA 95616, USA

MAMP-triggered immunity (MTI) has been traditionally implied to be involved in non-host resistance of *A. thaliana* (*At*) to *P. syringae* pv. phaseolicola 1448A (Pph1448A). However, Pph1448A produces number of effectors that can be potentially recognized and induce effector-triggered immunity (ETI) in *At* plants. In the attempt to determine the contribution of ETI to the interaction between *At* and Pph1448A we identified the patterns of effector recognition among different *At* ecotypes. We used Tobacco Rattle Virus (TRV)-based transient expression system to deliver effectors one-by-one and analyze their ability to induce ETI based on the phenotype of infected plants. We found that all three AvrB homologs present in Pph1448A trigger RPM1/RIN4- and TAO1-mediated defenses in Col-0. In addition two AvrB4 paralogs, but not AvrB2 trigger RPM1/TAO1-independent defenses in Col-0 due to RPS2 activation. An additional survey of other Pph1448A effectors revealed the ability of HopJ1 to trigger defenses in Col-0 and in a number of other ecotypes. Genetic mapping and reverse genetic approaches enabled us to link HopJ1-induced defenses to *Determinant of Effector Recognition 1 (DERK1*). *DERK1* is a single CC-NB-LRR encoding gene with no other known specificity reported to date. EDS1, NDR1, PAD4, RAR1 and SGT1a&b are not required for its function. We are pyramiding different knockouts in NB-LRR receptors to produce *At* lines compromised in Pph1448A effector recognition.

P82 Functional study of *Pseudomonas syringae* type III effector AvrE

X. XIN, K. NOMURA, F. URIBE, X. CHEN, S.-Y. HE

DOE-Plant Research Laboratory, Michigan State University, 612 Wilson Road and Rm222, East Lansing, MI, 48824, USA

The AvrE family of type III effectors is broadly conserved and arguably the most important virulence factors in phytobacteria, but their subcellular localization and molecular action inside the plant cell are not understood. We use the *Arabidopsis-Pseudomonas syringae* pv. *tomato (Pst)* DC3000 pathosystem to study AvrE function and have made several advances recently. First, we showed that two sequence motifs in AvrE, a WxxxE motif within the N-terminal half and a KK motif at the C-terminus, are essential for AvrE function. Second, co-expression of the N- and C-terminal halves of AvrE can reconstitute the cell death-inducing activity of wild type AvrE, and the two half proteins interact with each other in planta, suggesting AvrE is a modular protein and contains at least two functional domains. Third, YFP-AvrE protein has been localized to discrete puncta on the plasma membrane, and in vivo pull down using transgenically expressed AvrE as bait uncovered several candidate AvrE-interacting proteins that are defense-related and localized on membrane microdomain/lipid rafts. These new results raise the intriguing possibility that AvrE may exploit the membrane microdomain/lipid rafts as a platform to function as a virulence protein inside the plant cell.

P83 Functional characterization of a convergent host target of divergent pathogen effectors

LI YANG, PETRA EPPLE, JEFFERY L. DANGL

Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Pathogens employ a variety of weapons to antagonize the plant host immune system.
Effectors are pathogen proteins secreted into host cells where they interfere with host targets to achieve proliferation and ultimately dispersal of pathogens. Study of the interactions between effectors and their host targets is key to understanding the hostpathogen arms race. Members of the TCP transcription factor family, particularly TCP14, are heavily targeted by unrelated effectors from two evolutionarily divergent pathogens, *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis* (*Hpa*), indicative of their important roles in disease resistance. Although the developmental function of TCP14 has been well characterized, how it modulates plant defense is unknown. We find that TCP14 is required for full immunity against *Hpa*. TCP14 partially executes its immune function by promoting cytokinin-mediated defense. A putative model addressing how an *Hpa* effector blocks TCP14 function will be discussed.

P84 Genome evolution in an asexual lineage of *Phytophthora infestans*

KENTARO YOSHIDA, MARINA PAIS, LILIANA M. CANO, KAMIL WITEK, RICARDO F. OLIVA, VIVIANNE VLEESHOUWERS, SOPHIEN KAMOUN

The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

Phytophthora infestans, an oomycete capable of both sexual and asexual reproduction, rapidly adapts to new resistant potato cultivars. Successive emergence of asexual clonal lineages is a threat to potato cultivation. However, we know little about genome evolution in asexual plant pathogen lineages and the degree to which genetic or epigenetic variation alters pathogen virulence. The clonal lineage EC1 dominates South American populations of *P. infestans*. The *P. infestans* isolate EC1-3636 overcomes plant immunity via silencing of the RXLR effector gene *Avrvnt1* (Mathieu Pel, PhD thesis, Wageningen University). In contrast, a sibling isolate EC1-3527 expresses *Avrvnt1* and is avirulent on *Rpi-vnt1* potatoes. This finding suggests that asexual strains can evade effector-triggered immunity through modulation of RXLR effector gene expression. To determine which mechanisms drive adaptation of asexual *P. infestans* to host plants, we tested nucleotide diversity and expression polymorphisms of EC1-3626 and EC1-3527 using Illumina genome and transcriptome sequencing. We found that gene expression of several RXLR effectors, in addition to *Avrvnt1*, showed presence/absence gene expression polymorphisms between the EC1 strains. Modulation of gene expression of these RXLR effectors might be involved in adaptation to host plants and indicates rapid evolution of virulence traits within clonal lineages of *P. infestans*.

P85 The functions of the *Arabidopsis* TOP1 and TOP2 thimet oligopeptidases in salicylic aciddependent stress signaling

GIULIO ZAMPOGNA, MAGALI MOREAU, TIMOTHY WESTLAKE, MYAOING TAN, DAN KLESSIG, SORINA C. POPESCU

Boyce Thompson Institute for Plant Research, Cornell University, Tower Road, Ithaca, NY, 14853, USA

We have identified TOP1 protein in a high-throughput screen for proteins that interacted with a functional salicylic acid (SA) analog on *Arabidopsis* protein microarrays. Multiple complementary interaction assays have confirmed that SA interacts with both TOP1 and its predicted homolog, TOP2. *TOP1* and *TOP2* genes encode highly similar *Arabidopsis* thimet oligopeptidases. This project seeks to elucidate the cellular functions of *TOP1* and *TOP2* and their relationship with SA-dependent stress signaling. We have generated multiple single and double *Arabidopsis* mutant lines impaired in the expression of one or both *TOP* genes and have begun to exploring the roles of TOP1 and TOP2 in SA-dependent physiological responses activated following pathogen attack and other environmental stresses. *TOP1* and *TOP2* appear as key modulators of multiple SA-regulated signaling pathways including the immune-associated programmed cell death, maintenance of cellular redox homeostasis, and plant acclimation to photooxidative stress. Interestingly, TOP1 and TOP2 appear to perform similar but also distinct functions in SAdependent cellular signaling.

List of Speaker and Poster Abstracts

** S=speaker abstract; P=poster abstract; Presenting author abstract underlined*

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Participants

Jeff Anderson	University of Missouri	andersonjef@missouri.edu
Hagop Atamian	University of California-Riverside	hatam001@ucr.edu
Kyaw Joe Aung	Michigan State University	aungkyaw@msu.edu
Mark Banfield	John Innes Centre	mark.banfield@jic.ac.uk
Becky Bart	University of California-Berkeley	rbart@berkeley.edu
Friederike Bernsdorff	Heinrich Heine University	friederike.bernsdorff@uni-duesseldorf.de
Paul Birch	University of Dundee	Paul.Birch@hutton.ac.uk
Petra Boevink	The James Hutton Institute	petra.boevink@hutton.ac.uk
Adam Bogdanove	Cornell University	ajb7@cornell.edu
Jorunn Bos	The James Hutton Institute	jorunn.bos@hutton.ac.uk
Rick Bostock	University of California-Davis	rmbostock@ucdavis.edu
Marie Bourcy	ISV CNRS	marie.bourcy@isv.cnrs-gif.fr
Klaas Bouwmeester	Wageningen University	klaas.bouwmeester@wur.nl
Anita Brock	Leibniz-Institute of Vegetable Ornamental Crops	brock@igzev.de
Liliana Maria Cano Mogrovejo	The Sainsbury Laboratory	liliana.cano@tsl.ac.uk
Feng Yi Christine Cao	Univerisity of Toronto	feng.cao@utoronto.ca
Stella Cesari	INRA Montpellier	stella.cesari@supagro.inra.fr

Participants

Ritu Chaudhary	University of California-Riverside	rchau001@ucr.edu
Li Chen	Kochi University	s10dre16@s.kochi-u.ac.jp
Mi Sun Cheong	Stanford University	mcheong@stanford.edu
Gitta Coaker	University of California-Davis	glcoaker@ucdavis.edu
Yasin Dagdas	University of Exeter	yfd203@exeter.ac.uk
Jeff Dangl	University of North Carolina	dangl@email.unc.edu
Mara de Sain	University of Amsterdam	m.desain@uva.nl
Laurent Deslandes	University of Toulouse, LIPM	laurent.deslandes@toulouse.inra.fr
Darrell Desveaux	University of Toronto	darrell.desveaux@utoronto.ca
Savithramma Dinesh-Kumar	University of California-Davis	spdineshkumar@ucdavis.edu
Armin Djamei	Max Planck Institute for Terrestrial Microbiology	djamei@mpi-marburg.mpg.de
Peter Dodds	CSIRO Plant Industry	peter.dodds@csiro.au
Gunther Doehlemann	Max Planck Institute for Terrestrial Microbiology	doehlemann@mpi-marburg.mpg.de
Stéphan Dorey	University of Reims Champagne-Ardenne	stephan.dorey@univ-reims.fr
Seb Duplessis	INRA Nancy	duplessi@nancy.inra.fr
Jeff Ellis	CSIRO Plant Industry	jeff.ellis@csiro.au
J. Mitch Elmore	University of California-Davis	jmelmore@ucdavis.edu

Participants

Petra Epple	HHMI and UNC Chapel Hill	pepple@email.unc.edu
Adam Foster	Natural Resources Canada	Adam.Foster@RNCan-NRCan.gc.ca
Roger Freedman	Two Blades Foundation	rpf@mediant.co.uk
Koki Fujisaki	Iwate Biotechnology Research Center	k-fujisaki@ibrc.or.jp
Jorge Galán	Yale University	jorge.galan@yale.edu
Pam Gan	RIKEN	pamela.gan@psc.riken.jp
Martha C. Giraldo	Kansas State University	mgiraldo@ksu.edu
Laurence Godiard	University of Toulouse, LIPM	laurence.godiard@toulouse.inra.fr
Sandra Goritschnig	University of California-Berkeley	gosandra@berkeley.edu
Murray Grant	Exeter University	M.R.Grant@exeter.ac.uk
Ming Guo	University of Nebraska	mguo2@unl.edu
David Guttman	University of Toronto	david.guttman@utoronto.ca
Valentin Hammoudi	University of Amsterdam	v.hammoudi@uva.nl
Sheng-Yang He	Michigan State University	hes@msu.edu
Christoph Hemetsberger	Max Planck Institute for Terrestrial Microbiology	christoph.hemetsberger@mpi-marburg.mpg.de
Elizabeth Henry	University of California-Davis	ehenry@ucdavis.edu
Alexander Hof	Max Planck Institute for Terrestrial Microbiology	alexander.hof@mpi-marburg.mpg.de

Participants

Saskia Hogenhout	John Innes Centre	saskia.hogenhout@jic.ac.uk
Tram Hong	Max Planck Institute for Plant Breeding Research	hongngoctram@gmail.com
Edgar Huitema	University of Dundee	e.huitema@dundee.ac.uk
Muhammad Ilyas	Max Planck Institute for Plant Breeding Research	ilyas@mpipz.mpg.de
Joanna Jelenska	University of Chicago	jjelensk@uchicago.edu
David Joly	Pacific Agri-Food Research Centre	David.Joly@agr.gc.ca
Jonathan Jones	John Innes Centre	jonathan.jones@sainsbury-laboratory.ac.uk
Florian Jupe	The James Hutton Institute	florian.jupe@hutton.ac.uk
Julietta Jupe	University of Dundee	julietta.jupe@hutton.ac.uk
Clarence 'Cal' Kado	University of California-Davis	cikado@ucdavis.edu
Regine Kahmann	Max Planck Institute for Terrestrial Microbiology	kahmann@mpi-marburg.mpg.de
Sophien Kamoun	The Sainsbury Laboratory	sophien.kamoun@tsl.ac.uk
Hiroyuki Kanzaki	Iwate Biotechnology Research Center	hkanzaki@ibrc.or.jp
Kestur N. Amruthesh	University of North Texas	dr.knamruthesh@gmail.com
Graeme Kettles	University of California-Riverside	graeme.kettles@ucr.edu
Jung-Gun Kim	Stanford University	junggunk@stanford.edu
Sang Hee Kim	Indiana University	kim659@indiana.edu

Participants

Bostjan Kobe	University of Queensland	b.kobe@uq.edu.au
Anja Kombrink	Wageningen University	anja.kombrink@wur.nl
Ksenia Krasileva	University of California-Davis	krasileva@ucdavis.edu
Magdalena Krzymowska	Institute of Biochemistry and Biophysics	krzyma@ibb.waw.pl
Jiyoung Lee	University of Chicago	jiyoung1@uchicago.edu
Amy H Lee	University of Toronto	ahy.lee@mail.utoronto.ca
Donghyuk Lee	University of California-Davis	sdhlee@ucdavis.edu
Jennifer Lewis	USDA/University of California-Berkeley	jdlewis@berkeley.edu
Guangyong Li	University of Nebraska	gli3@unl.edu
Wei Li	University of California-Davis	wwwli@ucdavis.edu
Dan Lin	University of California-Davis	danlin@ucdavis.edu
Hong-Xia Liu	Stanford University	hxliu@njau.edu.cn
Timothy Lo	University of Toronto	timothy.lo@mail.utoronto.ca
Jose Lozano-Torres	Wageningen University	jose.lozano@wur.nl
Francis Martin	INRA Nancy	fmartin@nancy.inra.fr
Greg Martin	Cornell University	gbm7@cornell.edu
Paula Martins	UNESP	pmmm@rc.unesp.br

Participants

Magdalena Julita Mazur	University of Amsterdam	m.j.mazur@uva.nl
Hazel McLellan	University of Dundee	hazel.mclellan@hutton.ac.uk
Richard Michelmore	University of California-Davis	rwmichelmore@ucdavis.edu
Melissa G. Mitchum	University of Missouri	goellnerm@missouri.edu
Mary Beth Mudgett	Stanford University	mudgett@stanford.edu
Ji-Chul Daniel Nam	University of Missouri	jcnbm5@mail.missouri.edu
Nina Neidig	Max Planck Institute for Terrestrial Microbiology	nina.neidig@mpi-marburg.mpg.de
Benjamin Petre	INRA Nancy	Benjamin.Petre@nancy.inra.fr
Sorina Popescu	Boyce Thompson Institute for Plant Research	scp78@cornell.edu
Daniil Prigozhin	University of California-Berkeley	prigozhin@gmail.com
Matthew Pye	University of California-Davis	mfpye@ucdavis.edu
Maryam Rafiqi	University of Giessen	maryam.rafiqi@agrar.uni-giessen.de
Pascal Ratet	ISV CNRS	pascal.ratet@isv.cnrs-gif.fr
Thomas Redditt	Indiana University	tredditt@indiana.edu
Natalia Requena	Karlsruhe Institute of Technology	natalia.requena@kit.edu
Susana Rivas	University of Toulouse, LIPM	rivas@toulouse.inra.fr
Sara Roberts	University of California-Davis	smrobinson@ucdavis.edu

Participants

Hiromasa Saitoh	Iwate Biotechnology Research Center	saitoh@ibrc.or.jp
Diane Saunders	The Sainsbury Laboratory	diane.saunders@tsl.ac.uk
Shailendra Sharma	Iwate Biotechnology Research Center	shail6_r@rediffmail.com
Ken Shirasu	RIKEN	ken.shirasu@psc.riken.jp
Brian Staskawicz	University of California-Berkeley	stask@berkeley.edu
Nancy Stolle	Max Planck Institute for Terrestrial Microbiology	nancy.stolle@mpi-marburg.mpg.de
Will Stork	Stanford University	wstork@stanford.edu
Sudisha Jogaiah	Yamaguchi University	jsudish@gmail.com
Kathy Swords	LaVista Ag LLC	kmmswords@yahoo.com
Nick Talbot	University of Exeter	n.j.talbot@exeter.ac.uk
Ryohei Terauchi	Iwate Biotechnology Research Center	terauchi@ibrc.or.jp
Brett Tyler	Oregon State University	brett.tyler@oregonstate.edu
Huoi Ung	University of Toronto	huoi.ung@utoronto.ca
Fabienne Vailleau	University of Toulouse, LIPM	fabienne.vailleau@toulouse.inra.fr
Barbara Valent	Kansas State University	bvalent@ksu.edu
Peter van Esse	Wageningen University	peter.vanesse@wur.nl
Pieter van West	University of Aberdeen	p.vanwest@abdn.ac.uk

Participants

Claire Veneault-Fourrey	Université Lorraine / INRA	claire.fourrey@scbiol.uhp-nancy.fr
Anju Verma	University of Missouri	vermaan@missouri.edu
Jianying Wang	University of Missouri	wangjian@missouri.edu
Eric Ward	Two Blades Foundation	ericrward@gmail.com
Lennart Wirthmueller	John Innes Centre	Lennart.Wirthmueller@jic.ac.uk
Tadeusz Wroblewski	University of California-Davis	wroblewski@ucdavis.edu
Xiufang Xin	Michigan State University	xinxiufa@msu.edu
Li Yang	University of North Carolina	liyang2@live.unc.edu
Kentaro Yoshida	The Sainsbury Laboratory	Kentaro.Yoshida@tsl.ac.uk
Cyril Zipfel	The Sainsbury Laboratory	cyril.zipfel@tsl.ac.uk
Alga Zuccaro	Max Planck Institute for Terrestrial Microbiology	zuccaro.alga@mpi-marburg.mpg.de

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