Rust Workshop

29th Fungal Genetics Conference
Asilomar Conference Grounds
Pacific Grove, CA
March 14, 2017

Speakers

Cathy Aime
Sergio Brommonschenkel
Steve Whitham

Selected abstracts

Roshan Sharma Poudel
Jun Guo
Phil Tanguay
Cecile Lorrain

Panel Discussions

"Technologies; the good, the bad and the plain ugly"

Peter Dodds, Sebastien Duplessis and Benjamin Schwessinger

"Building community resources for genomics"

Diane Saunders, Melania Figueroa and Igor Grigoriev

http://www.genetics-gsa.org/fungal/2017/pages/workshops.shtml

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Fungal Genetics

Brief Program Outline

9:00 – 9:35 Suppression of PAMP-triggered immunity by *Hemileia vastatrix* candidate effectors. Sergio H. Brommonschenkel.

9:35 – 9:55 Rise to the bait: Towards identifying the *Puccinnia graminis* effector Avr4/5 "baited in" by the Rpg5 protein kinase integrated decoy domain. Roshan S. Poudel.

9:55 – 10:15 Host-induced gene silencing of a conserved and important pathogenicity factor MAPKK from *Puccinia striiformis* f. sp. *tritici* confers strong resistance to stripe rust in wheat. Jun Guo.

10:15– 10:45 Networking Break

10:45 – 11:05 RNA-seq analysis reveals host specific genes expressed by the white pine blister rust fungus *Cronartium ribicola* following infection on white pine and *Ribes*. Phil Tanguay.

11:05 – 11:40 Identification of *Phakopsora pachyrhizi* (Asian soybean rust) effector candidates that affect plant immune responses. Steve A. Whitham.

12:00 – 13:00 Lunch Break

13:00 – 13:35 Rust Phylogenetics. Cathy Aime

13:35 – 13:55 The poplar rust fungus effector biology: challenges of functional characterization of effectors in a non-model pathosystem. Cecile Lorrain.

14:00 – 15:15 Panel Discussion #1. "Published, unpublished, tried and failed experiments: towards successful identification and functional characterization of genes in rust fungi". Lead Panelists: Peter N. Dodds, Sebastien Duplessis, Benjamin Schwessinger.

15:15 – 15:45 Networking Break

15:45 – 17:00 Panel discussion #2: "Building genomic resources for the community". Lead Panelists: Diane G.O Saunders, Melania Figueroa, Igor Grigoriev.

17:00 – 17:20 Final remarks

Program outline and abstracts

Morning session

9:00 – 9:35 Suppression of PAMP-triggered immunity by *Hemileia vastatrix* candidate effectors T. Maia, G. Marin-Ramizez, D. Rezende, **Sergio H. Brommonschenkel**. Universidade Federal de Viçosa, Departamento de Fitopatologia, Brazil. *Corresponding author: shbromo@ufv.br

During the interaction with host plants, rust fungi secrete and translocate several effector proteins into the cytoplasm of plant cells to suppress defense responses. In order to understand the role of these effectors in the pathogenesis of coffee rust, this study aimed the functional analysis of candidate effectors from *Hemileia vastatrix*, by evaluating their ability to suppress PTI (PAMP-Triggered Immunity) responses triggered by the non-pathogenic bacteria *Pseudomonas fluorescens* EtHAn (PfEtHAn) as well as to determine their subcellular localization. Gene sequences encoding for 54 putative effectors were individually cloned into pEDV6 vector (without signal peptide) and mobilized to PfEtHAn for transient expression in *Nicotiana benthamiana*. Fifteen candidate effectors suppressed PTI with high reproducibility in different coinfiltration experiments of PfEtHAn with the pathogenic bacterium *P. syringae* pv. *tomato* DC3000. The suppression of PTI by two candidate effectors (HvEC-064 and HvEC-084) has been confirmed by analyzing DC3000 population growth in the infiltrated tissues where PTI was suppressed. To determine where the candidate effectors accumulate in plant cells, we cloned the coding sequences to obtain candidate effector-green fluorescent protein fusions in an *Agrobacterium tumefaciens* binary vector. The fusion proteins were transiently expressed in *N. benthamiana* leaf cells by agroinfiltration. These screens have revealed one *H. vastatrix* effector that accumulates in the nucleus of plant cell.

9:35 – 9:55 Rise to the bait: Towards identifying the *Puccinnia graminis* effector Avr4/5 "baited in" by the Rpg5 protein kinase integrated decoy domain. **Roshan S. Poudel[1]**, S. Solanki[1], S. Shrestha[1], J. Richards[1] and R. Brueggeman[1*]. [1]Department of Plant Pathology, North Dakota State University. *Corresponding author: Robert.brueggeman@ndsu.edu

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is a threat to wheat and barley production with virulent races posing a threat to world food security. The barley *rpg4/Rpg5* resistance locus (*rpg4/5*) confers resistance against many *Pgt* races, including the highly virulent race TTKSK (A.K.A Ug99) and its lineage. *Rpg5* is the functional resistance gene at this locus encoding a typical NLR with a predicted C-terminal protein kinase (PK), which may represent an integrated decoy domain supported by genomic architecture and predicted protein domain function. However, validation of this hypothesis is hampered without identification of the corresponding *Pgt* Avr4/5 effector. This research aims to identify and validate *Pgt Avr4/5* by utilizing 37 *Pgt* isolates showing a differential response on barley genotype with *rpg4/5*. These isolates were genotyped utilizing Restriction Site Associated DNA-Genotyping by Sequencing to identify 24 diverse isolates then *in planta* RNAseq was conducted on the susceptible barley variety Harrington, 5 days post inoculation with these 24 diverse isolates. Eleven candidate secreted effector proteins (CSEPs) showing differential expression (presence/absence) correlating with avrrpg4/5 and AvrRpg4/5 isolates were identified. These CSEPs are being transformed into *Pichia pastoris* for protein expression and infiltration in barley *rpg4/5*+ and *rpg4/5*- genotypes. These CSEPs are also being tested in yeast-two-hybrid interaction with the Rpg5 PK domain as bait. A mutation approach involving gamma irradiation of seven AvrRpg4/5 isolates to induce virulence by mutating the *Avr4/5* gene in these isolates is also being utilized towards Avr4/5 identification.

9:55 – 10:15 Host-induced gene silencing of a conserved and important pathogenicity factor MAPKK from *Puccinia striiformis* f. sp. *tritici* confers strong resistance to stripe rust in wheat. Jun Guo*, Xiaoguo Zhu, Zhensheng Kang. State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, P. R. China *Contact Email: guojunwgq@nwsuaf.edu.cn

Rust fungi are disastrous and notorious plant pathogens. Utilization existing germplasm resources to accelerate genetic breeding to control the diseases is particularly urgent due to the rapid evolution of rust fungi. A widely used powerful genetic tool, RNA interference (RNAi), has been used as a method to achieve durable resistance of plants to fungal pathogens. Here, we reported the MAPK kinase, PsFUZ7, which was proved to be an important pathogenicity factor in *Puccinia striiformis* f. sp. *tritici* (Pst), and had been selected as the target to construct the stable small RNA interfering materials for wheat stripe rust control. Transgenic wheat analyses indicated that stable silencing *PsFUZ7* functions profitably to suppress infection and development of Pst. Further results revealed that the transcript levels of some MAPK pathway-related genes in Pst were decreased, whereas some defense-related genes in plants increased. Our results indicate that host-induced gene silencing of an important fungal MAPKK gene is an effective strategy for durable control of cereal rust.

10:15– 10:45 Networking Break

10:45 – 11:05 RNA-seq analysis reveals host specific genes expressed by the white pine blister rust fungus *Cronartium ribicola* following infection on white pine and *Ribes*. **Phil Tanguay,** A. J. Foster, B. Dillon, N. Feau, R. C. Hamelin. Natural Resources Canada, Laurentian Forestry Centre, Quebec. Contact Email: philippe.tanguay@canada.ca

Cronartium ribicola is the rust fungus responsible of white pine blister rust, a lethal disease on North American 5-needles pines. *C. ribicola* is a macrocyclic heteroecious fungus which alternates between gymnosperm (5-needles pines) and angiosperm (mainly *Ribes spp.)* hosts. We hypothesize that the pathogen expresses different genes during infection and colonization of these two divergent hosts. We performed RNA-seq analysis and compared expression of *C. ribicola* genes from infection on pine stems and needles, and *Ribes* leaves. Comparison of transcriptomes from revealed set of *C. ribicola* genes differentially expressed genes and specifically associated with fungal infection on the aecial (pine) and telial (*Ribes*) hosts. Our global gene expression profiling presents a comprehensive view of transcriptomic regulation in the WPBR pathosystem and yields novel insights on molecular and biochemical mechanisms involved in infection by *C. ribicola* of its two divergent hosts.

11:05 – 11:40 Identification of *Phakopsora pachyrhizi* (Asian soybean rust) effector candidates that affect plant immune responses. M. Qi and **Steve A. Whitham*** . Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA USA. *Corresponding author: swhitham@iastate.edu

We previously sequenced the haustorial transcriptome of the Asian soybean rust fungus, *Phakopsora pachyrhizi*, and nearly 150 putatively secreted proteins were predicted. We called these proteins *P. pachyrhizi* effector candidates (*Pp*ECs), and 82 of the longest full-length coding sequences were cloned into various vectors that enabled bacterial delivery via the Type III secretion system, viral delivery, transient expression via *Agrobacterium* infiltration, or expression in yeast. Given the challenges of investigating effector functions directly in this obligate, biotrophic fungus, we have used these systems as surrogates to identify several *Pp*ECs that have abilities to suppress and/or activate defense or defense-like responses in host and non-hosts. We will describe these systems and provide an overview of the results for the 82 *Pp*ECs. The *Pp*ECs were also tagged with GFP so that their possible localizations within plant cells could be determined. We will then describe further characterization of *Pp*EC15, a protease that attenuated basal defense in tobacco and Arabidopsis and was localized to the nucleus when transiently expressed in *Nicotiana benthamiana*. The results indicate that *Pp*EC15 cleaves one or more nuclear plant proteins that positively regulate basal immunity.

12:00 – 13:00 Lunch Break

Afternoon session

13:00 – 13:35 Rust Phylogenetics. Cathy Aime *Corresponding author: maime@purdue.edu

13:35 – 13:55 The poplar rust fungus effector biology: challenges of functional characterization of effectors in a non-model pathosystem. **Cecile Lorrain[1]**, B. Petre[1], D. G.O. Saunders [π], J. Sklenar[£], J. Win[£], S. Kamoun[£], C. Delaruelle[1], J. Petroswky[1], P. Frey[1], A. Hecker[1], S. Duplessis[1*]. [1] UMR 1136 INRA/Université de Lorraine Interactions Arbres/Microorganismes, Centre INRA Nancy Lorraine, F-54280 Champenoux, France; [π] Present address, Earlham Institute, Norwich Research Park, Norwich, United Kingdom; [£] Present address, The Sainsbury Laboratory, Norwich Research Park, Norwich, Unite Kingdom. *Corresponding author: sebastien.duplessis@inra.fr

Rust fungi are devastating biotrophic pathogens manipulating host processes by delivering effector proteins into the plant cells. The poplar leaf rust fungus *Melampsora larici-populina* genome analysis revealed a large set of secreted proteins that some have been considered as candidate effectors. The understanding how these effector proteins function in the host cells has been the key question of effector biology for the last decade. Many efforts have been made in the field plant-microbe molecular interactions to unravel their role in the colonization of plant tissues. The poplar-poplar rust pathosystem although considered, as a genomic model in the study of tree-microbe interactions is actually non-model pathosystem when it comes to functional characterization of effectors. Absence of easy-going transformation systems for poplar and rust fungi is a major drawback. However, we combined several tools and approaches to help at elucidate the roles of *M. larici-populina* effector proteins such as heterologous *in planta* expression, recombinant protein production or structural approach. All these diverse approaches have led to partially unravelling the role of numerous *M. larici-populina* effector candidates. Summary report of the ongoing research aimed at elucidating candidate effectors functions will be presented, rising questions about the methodological limits of the study of effector biology in non-model pathosystems.

14:00 – 15:15 Panel Discussion #1. "Published, unpublished, tried and failed experiments: towards successful identification and functional characterization of genes in rust fungi". Lead Panelists: Peter N. Dodds[1], Sebastien Duplessis[2], Benjamin Schwessinger[3]. [1]CSIRO Canberra, Australia, [2] UMR 1136 INRA/Université de Lorraine Interactions Arbres/Microorganismes, Centre INRA Nancy Lorraine, F-54280 Champenoux, France, [3] Australian National University, Canberra, Australia· Contacts: Peter.Dodds@csiro.au; sebastien.duplessis@inra.fr; benjamin.schwessinger@anu.edu.au

Rust fungus genome sequences are being generated at a fast pace and have provided a basis for breakthrough research for these obligate plant pathogens. However, identification of genes, categorization and functional analyses are still very challenging. We'll introduce some of the current ways for gene identification through comparative analyses, transcriptomes and proteomics; this includes subsets of pathogenicity / virulence genes and effectors, that is, (predicted) secreted proteins. The search for pathogenicity or (a)virulence genes has received much interest and current approaches include GWAS, genetic map-based identification in a segregating population, or sequencing and comparative analysis of natural or induced avr-to-vir mutants. What are the current ways for gene function analysis: analysis of natural or induced mutants, Host Induced Gene Silencing, complementation in heterologous microbial systems, homologous or heterologous (*N. benthamiana*, Arabidopsis, Yeast) plant systems: stable and transient expression systems for localization studies (fluorescent chimers), finding putative host targets / partners (through Y2H, BiFC & microscopy, or biochemically by co-IP), HR assays (biolistics, *Pseudomonas, Xanthomonas, Ustilago*, BSMV, etc.), cell-death suppression, host+pathogen transcriptomics to reveal gene networks by association, indirect by assaying other responses related to PTI (e.g., defining a common set of plant defense response genes). Some procedures exist, but a stumbling block is still a lack of routine genetic transformation protocols. Recent progress may include the use of *Agrobacterium*-mediated transformation, cell-penetrating peptides and particle bombardment. Are there species that are culturable? We have vast collections of cereal rusts…. what about availability of other rusts? The goal of this part of the workshop is to, after having discussed various technical issues that have been tried, to share information and get feedback on published and unpublished experiments, in particularly on tried but failed experiments conducted in various labs on various rust pathosystems. This includes transformation, effectors, signalling, mating/sex, etc.) and we would like to hear about tools and approaches of importance, but that may not be so easily applicable to any given system and from which the community could benefit by learning what is currently done, has been tried, but was not deemed successful and therefore was never published.

15:15 – 15:45 Networking Break

15:45 – 17:00 Panel discussion #2: "Building genomic resources for the community". Lead Panelists: Diane G.O Saunders[1], Melania Figueroa[2], Igor Grigoriev[3]. [1] Earlham Institute, Norwich Research Park, Norwich, United Kingdom, University of Minnesota, St. Paul, MN, USA, [3]The U.S. Department of Energy Joint Genome Institute, Walnut Creek, CA, USA. Contacts: Diane.Saunders@earlham.ac.uk; figue031@umn.edu; IVGrigoriev@lbl.gov

Rapid advances in next-generation sequencing (NGS) technologies over the past decade have provided new opportunities to expand our knowledge base in the field of rust biology. While traditional approaches such as genetic transformation have proven difficult for some rust species, enhancement in genomic resources provide new avenues to rapidly advance this field. For instance, the acquisition of genomic information has enabled comparative studies that have given new insight into important biological aspects of rust fungi such as evolution and genetic variation. By combining whole genome sequencing with recent algorithmic advances we now have the potential to generate high-quality rust genome assemblies. In addition, NGS applications branch to studies based on targeted sequencing, transcriptomic profiling of messenger and noncoding RNAs, chromatin immunoprecipitation sequencing, etc. Each of these studies creates large datasets that demand infrastructure, training and resources to translate big data into meaningful biological discoveries. During this panel discussion, we will provide a forum to discuss (i) on-going large-scale sequencing efforts within the rust community, (ii) possible applications of current state-of-the art sequencing technologies, (iii) the challenges associated with deposition, sharing and usage of existing or new datasets, and (iv) the need to enhance genomic resources and develop suitable tools/pipelines to extract biological information. Our aim is that such discussions will identify pathways to make data available open source and direct our efforts towards building resources in genomics that serve the entire community.

17:00 – 17:20 Final remarks. Guus Bakkeren[1] and Sebastien Duplessis[2]. [1] Agriculture and Agri-Food Canada, Summerland, BC, Canada V0H 1Z0, [2] UMR 1136 INRA/Université de Lorraine Interactions Arbres/Microorganismes, Centre INRA Nancy Lorraine, F-54280 Champenoux, France.· Contacts: Guus.Bakkeren@agr.gc.ca; sebastien.duplessis@inra.fr

Recap and the way forward as a collaborative community.