Nutritional factors modulating plant and fruit susceptibility to pathogens

AN INTERNATIONAL WORKSHOP

Sponsored by BARD

United States-Israel Binational
Agricultural Research and Development Fund

PROGRAM AND ABSTRACTS

Haifa, Israel February 25 and 26, 2018

Nutritional factors modulating plant and fruit susceptibility to pathogens

First Day of BARD Workshop

25 February 2018, SUNDAY

9:00-18:00 at the Technion Neve Shaanan campus - room 300, Biotechnology and Food Engineering

49 participants

8:00 Buses leave Haifa hotels participating in ECFG14

8.45-9:00 *Early Coffee*

9:00-9:30 Welcome Session

9:00-9:15 Benjamin A. Horwitz, Richard Wilson and Dov Prusky- greetings

9:15-9:30 BARD representative – Greeting

9:30-12:30 Session A – Primary metabolic strategies modulating fungal development and function of infection structures

Chair: Howard Judelson and Benjamin A. Horwitz

09:30-09:55 **Richard Wilson**, Metabolic strategies governing *Magnaporthe oryzae* growth in rice cells.

09:55-10:20 **Howard Judelson,** Host and lifestyle influence nutritional strategies of Phytophthora and Pythium

10:20-10:45 **Jin-Rong Xu**, Ammonium permeases and regulation of DON biosynthesis in *Fusarium graminearum* 10:45-11:05 *Coffee break and group photo*

11:05-11:25 **Benjamin A. Horwitz** *Cochliobolus heterostrophus* and maize: Iron homeostasis and virulence

11:25-11:50 **Burt Bluhm,** XK1 regulates carbon catabolism, sporulation, fumonisin B_1 production and pathogenesis in *Fusarium verticillioide*s.

11:50-12:20 **PANEL DISCUSSION**: Howard Judelson and Richard Wilson

12:20- 13:20 Lunch

13:20-15:20 Session B – Carbon and Nitrogen regulation of pathogenicity

Chair: Miguel Peñalva and Antonio di Pietro

13:20-13:45 **Eduardo Espeso,** Roles in pathogenicity of regulatory systems mediating response to alkaline pH

13:45-14:10 **Dov Prusky**, Carbon regulation of environmental pH and its effect on Pathogenicity and mycotoxin production

14:10-14:35 **Tânia Fernandes Ribeiro**, Intracellular pH controls fungal MAPK signaling and pathogenicity

14:35-15:00 **Miguel Peñalva,** On why fungal hyphae grow so fast by apical extension

15:00-15:20 **PANEL DISCUSSION: Antonio di Pietro and Miguel Peñalva**.

15:20- 15:40 Coffee Break

Session C 15:30-18:00: **Metabolic pathways affecting fungal lifestyle**

Chair: Robert Fluhr and Martijn Rep

15:40-16:05 **Martijn Rep**, Metabolism and pathogenicity in *Fusarium oxysporum*: what do we know?

16.05-16:30 **Timothy Chaya and Nicole Donofrio**, Utilizing confocal microscopy to dissect the role of reactive oxygen species in the pathogenesis of *Magnaporthe oryzae* and *Cochliobolus heterostrophus*

16:30-16:55 **José Minguez-Diaz**, Modulation of virulence in *Fusarium oxysporum*

16:55-17:20 **Shay Covo,** The NAD salvage pathway, a potential target for pesticide development - cut the supply when nutrient flow is limited

17:20-17:45 **Regine Kahmann,** An amazing treasure box: Core effectors in smut fungi and their regulation

17:45-18:00 PANEL DISCUSSION: **Robert Fluhr and Martijn Rep**

Joining the ECFG14 meeting

18:00-19:30	ECFG14 welcome reception, Churchill Auditorium
19:30-20:00	Opening Remarks
20:00-20:45	Keynote lecture: Arturo Casadevall
21:00	Buses to Haifa Hotels

Second Day of BARD Workshop

26 February 2018, Monday

Technion Faculty of Medicine (Green Lecture Hall)

14.30-15:30 Session D: Nutritional factors and pathogens of fruits

Chair: Richard A Wilson and Dov Prusky

14:30 -14:45 **Noam Alkan,** Anthocyanin and flavonoids role in pathogenicity of fruit pathogens

14:45 -15:00 **Edward Sionov**, Host factors modulating *Aspergillus carbonarius* pathogenicity in grapes

15:00 -15:20 **Gustavo H. Goldman,** Crosstalk between the mitogen activated protein kinase SakA^{HOG1}–MpkC and protein kinase A connects carbohydrate mobilization to cell wall biosynthesis

15.20-15.30 **Short Coffee Break**

15:30-18:30 Session E. ECFG14 Concurrent Session: Physiology and Metabolism

Chair: Richard A. Wilson and Dov Prusky

15.30-15:55 **Nick Talbot**, Investigating the biology of plant tissue invasion and cell-to-cell movement by the rice blast fungus *Magnaporthe oryzae*

15:55 -16:20 **Michael Thon,** Evolution of host range is associated with carbohydrate and protein metabolism

16:20 -16:45 **Martin Tegelaar,** Functional distinction of hyphal compartments

16:45 -17:15 *Coffee break (together with ECFG)*

17:15 -17:40 **Lars Voll,** Foliar sugar accumulation enhances priming of the salicylic acid-mediated defense response

17:40-18:05 **Richard B. Todd,** Branched chain amino acid biosynthesis genes and regulators in Aspergillus.

18:05-18:30 **Benz J. Phillip,** A taste for sour sugars: characterization of a highly efficient D-galacturonic acid metabolism in basidiomycetes

18:30-19:00 *Tea (BARD workshop only)*/ **DISCUSSION PANEL AND WORKSHOP SUMMARY: Nutritional factors in infection and new strategies for the control of fungal disease.**

MODERATORS: Richard A. Wilson, Dov Prusky, Robert Fluhr and Miguel Peñalva, Regine Kahmann

19:10 – bus to workshop dinner at a Druse Village and return to Haifa hotels

JOINT DINNER FOR ALL THE MEMBERS OF THE WORKSHOP

Abstracts

February 25

Session A – Primary metabolic strategies modulating fungal development and function of infection structures

Metabolic strategies governing *Magnaporthe oryzae* growth in rice cells.

Guangchao Sun[1], Christian Elowsky[2], Richard A. Wilson[1]

[1]Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, Nebraska

[2]Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, Nebraska

Magnaporthe oryzae is a filamentous ascomycete that causes devastating rice and wheat loss on a global scale. For the first few days of infection, the fungus proliferates in and between living rice cells without eliciting responses from the host. This is achieved by the deployment of secreted effectors that target plant defense pathways, and by the activation - in response to nutrient availability - of *M. oryzae* antioxidation systems that neutralize the host oxidative burst. Given that, in order to establish the biotrophic growth stage, plant innate immunity must not be triggered, we seek to understand the molecular decision-making processes that coordinate fungal growth with sustained plant defense suppression or avoidance in the host cell. Here, we will show how the integrity of the biotrophic interfaces between fungal invasive hyphae (IH) and the rice cell cytoplasm –the sites of effector deployment - is dependent on the autophagy branch of the *M. oryzae* TOR nutrient-signaling pathway. TOR is a conserved pathway in eukaryotes that controls cellular metabolism and development in response to available nutrients and energy. We have shown how inactive TOR signaling in *M. oryzae* drives the formation of the specialized appressorial infection cell on the nutrient-free host surface. Conversely, a Tps1-dependent metabolic shift to glucose metabolism following rice cell ingress activates TOR signaling in an ATP-dependent manner and is required for very early biotrophic growth. Considering the importance of TOR to infection-related development, we initiated a forward genetic screen to select for rapamycin resistant *M. oryzae* mutants that might identify new TOR signaling components. In this manner, we discovered *IMP1* encoding a previously unknown integral membrane protein required for TOR-autophagy branch signaling and rapamycin sensitivity. Under axenic growth conditions, $Imp1^{GFP}$ localized to

vacuoles and was shown to be required for controlling vacuole acidity, autophagic flux and vesicle trafficking in response to TOR signaling. *IMP1* was also required for glucose-dependent V-ATPase assembly. *In planta*, in addition to vacuoles, Imp1^{GFP} associated with the IH membrane. Δ*imp1* strains could access host cells and elaborate IH, but became attenuated for biotrophic growth. This was accompanied by both the expulsion of the fluorescent apoplastic effector Bas4^{GFP} into the rice cytoplasm, and loss of the cytoplasmic effector-accumulating biotrophic interfacial complex (BIC), together indicative of biotrophic interface integrity failure. Inhibiting autophagy in WT during biotrophy recapitulated Δ*imp1*. Treating Δ*imp1* strains with the TOR-independent autophagy stimulator amiodarone hydrochloride remediated membrane integrity, effector secretion and biotrophic growth. We conclude that the novel TOR-Imp1-autophagy signaling axis integrates nutrient signals to maintain biotrophic interface integrity and function, control effector secretion and promote fungal growth in rice cells. The significance of our results lies in unexpectedly revealing how fungal metabolic status dictates rice –*M. oryzae* biotrophic interface longevity.

Cochliobolus heterostrophus and maize: Iron homeostasis and virulence

Shinichi Oide[1], Bradford J Condon[1], Ning Zhang[1], Benjamin A. Horwitz[2] and B. Gillian Turgeon[1]

[1]Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science in the College of Agriculture and Life Sciences, Cornell University, Ithaca, NY, USA and [2]Faculty of Biology, Technion – Israel Institute of Technology, Haifa 3200000, Israel.

Iron is an essential nutrient, while excess soluble iron contributes to oxidative stress by promoting formation of hydroxyl radicals by the Fenton reactions. Uptake, transport and sequestration of iron is tightly regulated. Plants withhold iron from their pathogens, while the pathogens have evolved high-affinity uptake systems [1]. Loss of genes for siderophore biosynthesis, for example, often results in decreased fitness of pathogens. *NPS6*, encoding the nonribosomal peptide synthetase for the extracellular siderophore, is needed for full virulence of *Cochliobolus heterostrophus* on maize. Siderophore mediated iron uptake rather than reductive iron assimilation is the preferred mechanism in this pathogen [2]. Deletion of *NPS6* results in loss of extracellular siderophore biosynthesis, attenuated virulence, hypersensitivity to oxidative and iron-depletion stress, and reduced asexual sporulation, while *nps2* mutants are phenotypically wild type in all of these traits but defective in sexual spore development when *NPS2* is missing from both mating partners [2]. Loss of the gene *SRE1*, encoding a GATA transcription factor siderophore biosynthesis repressor, altered sensitivity to iron, oxidative stress, and virulence to the host. Double mutants lacking *NPS6* and the redox-sensitive transcription factor ChAP1 were more sensitive to oxidative stress than either *Chap1* or *nps6* single mutants, while *Chap1sre1* double mutants showed a modest increase in resistance to oxidants as compared with single *Chap1* mutants but were much more sensitive than *sre1* mutants. These findings suggest that the NPS6 siderophore indirectly contributes to redox homeostasis via iron sequestration, while Sre1 misregulation may render cells more sensitive to oxidative stress. The double-mutant phenotypes are consistent with a model in which iron sequestration by NPS6 defends the pathogen against oxidative stress [3]. If manipulation of iron metabolism in plants is to provide a strategy against fungal pathogens, the key to application may lie in the balance between withholding Fe from the pathogen, and providing Fe to assist the plant in its oxidative defense. Data from *C. heterostrophus* are continuing to provide insight into this question.

[1] Verbon EH, Trapet PL, Stringlis IA, Kruijs S, Bakker P, Pieterse CMJ: Iron and Immunity. *Annu Rev Phytopathol* 2017, 55:355-375

[2] Condon BJ, Oide S, Gibson DM, Krasnoff SB, Turgeon BG: Reductive iron assimilation and intracellular siderophores assist extracellular siderophore-driven iron homeostasis and virulence. *Molecular plant-microbe interactions : MPMI* 2014, 27(8):793-808.

[3] Zhang N, MohdZainudin NA, Scher K, Condon BJ, Horwitz BA, Turgeon BG: Iron, oxidative stress, and virulence: roles of iron-sensitive transcription factor Sre1 and the redox sensor ChAp1 in the maize pathogen Cochliobolus heterostrophus. *Molecular plant-microbe interactions : MPMI* 2013, 26(12):1473-1485.

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Ammonium permeases and regulation of DON biosynthesis in *Fusarium graminearum*

Rui Hou and Jin-Rong Xu

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The wheat head blight pathogen *Fusarium graminearum* not only causes severe yield losses but also often contaminates infested grains with deoxynivalenol (DON), a trichothecene mycotoxin inhibitory to protein synthesis in eukaryotes. Deletion of the AreA ortholog significantly reduced DON production and affected the suppression of *TRI* gene expression by ammonium or stimulation of DON biosynthesis by arginine. Interestingly, the Δ*areA* mutant was reduced in the expression of all three predicted ammonium permease (*MEP*) genes under low ammonium conditions. Phenotype characterization of the mutants deleted of individual *MEP* genes suggested that *MEP2* functions as the most important ammonium permease in *F. gramniearum*. However, the *mep2 mep3* double mutant had more severe defects than the *mep2* mutant, including a reduced Gpmk1 phosphorylation level. Deletion of *MEP2* diminished the repression of *TRI* gene expression by ammonium. Expression of the wild-type *MEP2* but not the *MEP2* allele truncated of the C-terminal cytoplasmic region fully complemented the *mep2* deletion mutant. Mep2 likely functions as an ammonium sensor in *F. graminearum* and its C-terminal region may be directly involved in intracellular signaling

Host and lifestyle influence nutritional strategies of *Phytophthora* and *Pythium*

Howard S. Judelson

Department of Microbiology and Plant Pathology, University of California, Riverside USA

Plant pathogens exhibit lifestyles ranging from biotrophy to necrotrophy. The contribution of effector-mediated suppression of host defenses to biotrophy is well-established. We hypothesize that biotrophy and necrotrophy are also reflected in distinct strategies for metabolism and nutrient uptake. To explore this, we are studying the oomycetes *Phytophthora infestans* and *Pythium ultimum* during their colonization of potato tubers. *Ph. infestans* is a largely biotrophic pathogen that feeds from living host cells, which become necrotic only late in infection. In contrast, *Py. ultimum* is a necrotroph that macerates host tissues. Genome-wide analyses indicate that the metabolic capabilities of the two species are similar, with only a few exceptions. Many metabolic genes belong to families that often vary in size between the species, although impact of family expansions is usually tempered by mechanisms that suppress transcription of the additional genes. Nevertheless, the expression levels of many metabolic pathways differ between the species, especially during infection. For example, the fraction of mRNA devoted to lipid and starch metabolism in infection is greater in *Py. ultimum,* while amino acid and sucrose metabolism is higher in *Ph. infestans.* This may be explained by the fact that while *Py. ultimum* can access all plant metabolites, *Ph. infestans* is limited to the subset that exit the living cells of its host, requiring more metabolic transformations. The delivery of enzymes such as invertase to *Ph. infestans* haustoria support that organ's role in nutrition, although comparative studies with *Py. ultimum* (which lacks haustoria) present few candidates for haustoria-specific transporters. Differences between the oomycetes were seen in their use of nitrogen sources. Isotopic labelling studies indicated that *Py. ultimum* makes greater use of nitrate, which may be an adaptation that allows it to grow both as a pathogen and a saprophyte in soil.

Comparative and Functional Genomics Approaches to Dissect the Nutritional Regulation of Cercosporin Biosynthesis Among *Cercospora* spp.

Burt H. Bluhm
University of Arkansas – Fayetteville, USA

With over 3000 named species, *Cercospora* is one of the largest genera of fungal foliar pathogens. Many *Cercospora* species produce cercosporin, a polyketide-derived perlyenequinione that functions as a non-host-specific phytotoxin. Although a ~50 kb gene cluster underlying cercosporin biosynthesis (designated *CTB*) is broadly conserved among cercosporin-producing fungi, the nutritional regulation of cercosporin biosynthesis is hypothesized to vary among *Cercospora* species. Utilizing a blend of comparative and functional genomics, the nutritional regulation of cercosporin biosynthesis was explored in *Cercospora* pathogens of maize and soybean. Among *Cercospora* species with differing nutritional requirements for cercosporin production *in vitro*, the *CTB* cluster was highly conserved, yet a subset of *CTB* genes was differentially expressed in non-producers. In *C. zeae-maydis*, the causal agent of gray leaf spot of maize, a putative ortholog of the nitrogen response regulator *areA* was implicated in the regulation of cercosporin biosynthesis. Intriguingly, deletion of *CTB1*, which encodes the polyketide synthase within the *CTB* cluster, blocked *in vitro* cercosporin production in *C. zeae-maydis*, yet deletion strains retained wild-type levels of pathogenesis on maize leaves. Preliminary results suggest one or more additional polyketide synthases exclusively expressed *in planta* synthesize polyketide precursors of cercosporin during pathogenesis. A forward genetic screen coupled with a novel target-enrichment sequencing approach to characterize T-DNA insertion sites identified candidate regulatory genes outside of the *CTB1* cluster, some of which were implicated in nutrient sensing and/or acquisition. Collectively, these findings suggest an important nutritional component to the regulation of cercosporin biosynthesis *in planta*, which could serve as a checkpoint during the transition from hemibiotrophy to necrotrophy during host colonization.

Session B: Carbon and Nitrogen regulation of pathogenicity

Roles in pathogenicity of regulatory systems mediating response to alkaline pH

Eduardo A. Espeso.

Centro Investigaciones Biológicas. CSIC. Madrid.

Filamentous fungi colonize a wide range of environments and living hosts, including soil, plants, animals and humans. They are also capable of growing in extreme environmental niches surviving to various forms of stresses including osmotic stress, oxidative stress, nutrient deprivation, heat shock and changes in pH. To deal with with these variety of stresses, fungi have developed sophisticated regulatory mechanisms to alleviate these extracellular harsh conditions.

Our laboratory has been focused on the understanding of how fungi generate an adequate response to ambient alkaline pH. We discovered that three regulatory systems coexist in the model *Aspergillus nidulans* and other filamentous fungi. Two of these systems are also involved in tolerance to excess of extracellular cations. Historically, we defined PacC as the major regulator of ambient pH signaling. More recently we described the calcineurin-dependent transcription factor CrzA as required for growth at alkaline pH and in conditions of high extracellular calcium concentrations and SltA as a factor required for tolerance to high alkali-cation concentrations and to alkalinity. CrzA and PacC homologues are present in almost all known fungal genomes, however SltA homologues can only be found in species of *Pezizomycotina* subphylum. All these transcription factors are subjected to posttranslational modifications modulating their activities. Particularly PacC and SltA are subjected to an irreversible PTM modification, the regulated proteolysis.

Some aspects of fungal pathogenicity relay on the capacity of fungi to prosper under ambient stresses. Work from our laboratory and others have pointed to the role of these three major regulatory systems on pathogenicity. Here I will describe previous findings in *Aspergilli* and our work on the Slt pathway aimed to decipher conservation of signaling mechanisms and the role of this pathway in pathogenicity of animals and plants.

Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity and secondary metabolites accumulation in phytopathogenic fungi

Dov Prusky

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Fruit pathogens can contribute to acidification or alkalization of the host environment. This capability has been used to divide fungal pathogens into acidifying and/or alkalizing classes. Here we show that diverse classes of fungal pathogens—*Colletotrichum gloeosporioides*, *Penicillium expansum*, *Aspergillus nidulans,* and *Fusarium oxysporum*—secrete small pH-affecting molecules. These molecules modify the environmental pH that dictates acidic or alkaline colonizing strategies and induce the expression of PACC-dependent genes. We show that in many organisms, acidification is induced under carbon excess. In contrast, alkalization occurs under conditions of carbon deprivation. The carbon source is metabolized by *gox2* to gluconic acid, contributing to medium acidification, whereas catalyzed deamination of non-preferred carbon sources, such as the amino acid glutamate, by *gdh2* results in the secretion of ammonia. Isogenic lines of tomato containing differential concentrations of sugar may affect as well fungal response and colonization. Sucrose concentration also modulated LaeA, the global regulator of secondary metabolites accumulation in *P. expansum*, suggesting the importance of sugar regulation of fungal metabolism and mycotoxin accumulation. The present results indicate that differential pH modulation by fruit fungal pathogens is a host-dependent mechanism, affected by host sugar content, which modulates environmental pH to enhance fruit colonization.

Intracellular pH controls MAPK signaling and pathogenicity

Tânia Ribeiro Fernandes[1], Antonio Serrano Salces[1], Teresa Fernández-Acero[2], David Turrà[1], María Molina[2], Antonio Di Pietro[1]

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[2] Departamento de Microbiología II, Universidad Complutense de Madrid, 28040 Madrid, Spain.

pH is a key player in the control of fungal pathogenicity. We previously found that extracellular pH governs pathogenicity in the plant pathogen *Fusarium oxysporum* by reprogramming phosphorylation levels of mitogen-activated protein kinases (MAPKs). The molecular events underlying the pH response are currently unknown. Here we identify intracellular pH (pH_i) as a key signal regulating MAPK activity in *F. oxysporum*. Using the ratiometric GFP-based pH sensor pHluorin, we found that *F. oxysporum* responds to extracellular alkalinisation and acidification with a transitory shift in pH_i. Exogenous application of diethylstilbestrol (DES), a specific inhibitor of the plasma membrane H^+-ATPase Pma1, induced a rapid and sustained decrease of pH_i accompanied by rapid and transitory changes in MAPK phosphorylation, supporting the idea that pH_i acts as a key switch controlling MAPK activity. To search for fungal proteins involved in pH_i-mediated MAPK regulation, we screened a subset of acid-sensitive mutants from the yeast deletion library for loss of DES-triggered MAPK phosphorylation. This identified a number of candidates functioning in conserved cellular processes such as lipid metabolism, endocytosis or V-ATPase function, many of which have predicted orthologs in *Fusarium*. Understanding how pH_i regulates MAPK signaling may reveal new ways to control fungal growth, development and pathogenicity

On why fungal hyphae grow so fast by apical extension

Miguel A. Peñalva, Ignacio Bravo, Mario Pinar, Herbert N. Arst, Jr. and Miguel Hernández-González

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The vegetative phase of filamentous fungi consists of tube-shaped cells (hyphae) that grow exclusively by apical extension. Besides being a distinctive feature of hyphal organisms, polarized growth underlies the capacity of filamentous to explore substrates or, in the case of fungal pathogens, to invade tissues. To sustain the strikingly fast rates of growth that characterize fungi, the secretory pathway must efficiently deliver to the apex both the lipids accounting for the extension in plasma membrane surface and the enzymes that synthesize the cell wall in the hyphal tip dome. Our studies with a cell-wall modifying enzyme cargo have established that its polarization to the apical dome is mediated by endocytic recycling. This pathway streamlines the delivery of cargoes whose function is required at the tip to their locale of action, thereby facilitating rapid apical extension

PANEL DISCUSSION: Antonio di Pietro and Miguel Peñalva.

Session C: Metabolic pathways affecting fungal lifestyle

Metabolism and pathogenicity in *Fusarium oxysporum*: what do we know?

Martijn Rep

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

The *Fusarium oxysporum* species complex (FOSC) is notorious for harbouring plant-pathogenic clonal lines that cause wilt or root rot in crops and ornamentals. However, pathogenicity is in fact rare in the FOSC and *F. oxysporum* is commonly found in the rhizosphere and as endophyte in symptomless plants of many species. The induction of disease symptoms by a pathogenic clonal line of *F. oxysporum* is host-specific and is associated with extensive proliferation in roots and vascular tissue. We now know that this host-specific pathogenicity requires the secretion of a particular set of 'effector' proteins encoded on dispensable 'pathogenicity' chromosomes.

It may be expected that growth inside a living plant also requires particular metabolic capabilities, but much remains to be learned in this respect. We have obtained some information through insertional mutagenesis screens for loss or strong reduction of pathogenicity. From analysis of some of the mutants and the genes affected in these mutants we found indications that peroxisomes, secreted cell-wall degrading enzymes and degradation of aromatic compounds are important for pathogenicity. On the other hand, the glyoxylate cycle is apparently not important for pathogenicity.

Utilizing confocal microscopy to dissect the role of Reactive oxygen species in the pathogenesis of *Magnaporthe oryzae* and *Cochilobolus heterostrophus*

Chaya T[1,2], Donofrio NM[1], Cooper JG[1], Mikolajewski D[1], Neifert J[1], Huang, K[1] , Sweigard, JA[3], Wisser, R[1], Horwitz B[4], Caplan J[2]

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Reactive oxygen species (ROS) play important roles during plant-pathogen interactions, including the ability of a fungus to sense and withstand the ROS produced by plant defenses. While many studies have examined ROS responses between specific plant-fungal interactions, questions remain about how these responses compare across different fungal pathogens. In collaboration with the Horwitz lab, we aim to explore the mechanisms of ROS creation and responses in the hemi-biotroph *Magnaporthe oryzae* and the necrotroph *Cochilobolus heterostrophus*, which cause rice blast and Southern Leaf Blight, respectively. The infection cycle of both is being analyzed with advanced, three-dimensional (3D) confocal microscopy and image analysis to characterize the progression of these pathogens, using the Sca*l*eP technique to clear host leaves inoculated with their respective fungi. We captured data over a fourth dimension (4D), time, to show pathogenesis progression from germination to full infection into the host tissue. We also will utilize a genetically-encoded ROS sensor, called HyPer, in both *M. oryzae* and *C. heterostrophus* coupled with various ROS staining techniques in the host tissue. A time course of the HyPer sensor in *M. oryzae* (MoHyPer) shows the increase in ROS production through the early infection stages on the leaf surface. These tools will allow us to quantify the ROS response from both perspectives and make comparative analyses between ROS levels and time of infection in these two distinct pathosystems. In conjunction with these imaging techniques, we will build randomly mutagenized libraries in both fungi containing the HyPer sensor, and work has already begun in *M. oryzae*. A forward genetic screen will identify changes in ROS sensing/detection, and mutants of interest will be sequenced to determine the genes that have altered the ROS response. Data will be compared between the species, providing differences and similarities between necrotrophic and hemi-biotrophic ROS responses.

Partial deletion of the small chromosome produces loss of pathogenicity in *Fusarium oxysporum*

Virginia Casado-del Castillo[1], Riccardo Baroncelli[2], Michael R. Thon[1], Ernesto P. Benito[1], Jose M. Diaz-Minguez[1]

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[2]*Laboratoire Universitaire de Biodiversité et Écologie Microbienne, ESIAB, Université de Brest, Plouzané, France*.

Chromosome 14 is the smallest chromosome of the standard genome of *Fusarium oxysporum* (*F. oxysporum* f. sp. *lycopersici* strain 4287) and has been described as a 'pathogenicity chromosome'. It contains *loci* that encode virulence/pathogenicity factors and confers pathogenicity to non-pathogenic strains after its transfer from a pathogenic strain. Also, it has been recently shown that complete loss of this chromosome results in the loss of pathogenicity, although partial deletions that affect only supercontig 22 do not reduce virulence (Vlaardingerbroek *et al*., 2016). This chromosome is likely equivalent to the smallest chromosome of *F. oxysporum* f. sp. *phaseoli* (FOP).

The *FTF* gene family is composed of two pathogenicity factors: *FTF1*, with multiple paralogues all located in the small chromosome of highly virulent strains of FOP, and *FTF2*, a single copy factor located in the core genome. Both factors are involved in virulence/pathogenicity (Niño-Sánchez *et al*., 2016). We describe here the isolation and characterization of some strains carrying a partial deletion of the small chromosome (FOP-SP1sChr-pΔ), as shown by the electrophoretic karyotypes analysis. We have sequenced the complete genome of one of the mutants. Alignment with the wild-type genome (FOP-SP1) shows that missing regions are spread in several contigs, and none of them fit with conserved chromosomes in wild-type *Fusarium* spp. The region deleted includes all the paralogues of *FTF1*. FOP-SP1sChr-pΔ mutants show a complete loss of pathogenicity on common bean plants, suggesting that the deleted fragment harbours the relevant set of genes required to produce disease in this forma specialis. Although no Fusarium wilt symptoms were observed in common bean plants inoculated with FOP-SP1sChr-pΔ mutants, confocal laser microscopic analysis revealed the ability of these strains to colonize the host, indicating a behaviour similar to that shown by endophytic strains.

Niño-Sánchez *et al*., 2016. Mol. Plant Pathol. *17*, 1124–1139. Vlaardingerbroek *et al*., 2016. Mol. Plant Pathol. *17*, 1455–1466.

The NAD salvage pathway, a potential target for pesticide development - cut the supply when nutrient flow is limited

Daniel Waiger, Gautam Anand, Yael Almog and Shay Covo

Department of Plant Pathology and Microbiology, Robert H. Smith Faculty of Agriculture. Hebrew University

Nicotine-Adenine Dinucleotide, NAD, is one of the most important molecules in the cell. It is part of many redox reactions; the balanced between its oxidized and reduced forms determines cell metabolism. In recent years it became apparent that the redox status of NAD is only part of the picture. NAD is consumed by several classes of enzymes, especially sirtuins histone deacetylases. Hyper consumption of NAD by these enzymes resulted in metabolic abnormalities. There is very little information regarding the NAD pathway in fungal plant pathogens. NAD can be generated de novo from tryptophan or recycled from NAM the byproduct of sirtuins. It was previously shown that NAM can inhibit the growth of *Candida albicans*. We were able to show that NAM can inhibit the growth of Fusarium oxysporum on PDA plates and tomato fruits. Interestingly, Hyphal inhibition was more significant that inhibition of conidia germination. The cellular response to NAM is by increasing the amounts of over 100 transcription factors, indicating the chemical disrupts proper transcription. To gain insight to the consumption of NAD during fungal development we measured its levels in germinating conidia in water where no external nutrients are available. There is very little change in the total amounts of NAD although the NAD/NADH ratio is reduced. The same occurs when the NAD salvage pathway is targeted by nicotinaldehyde that was shown to bind tightly Pnc1. This change in NAD redox status is in agreement with the transcriptomics changes occur in the cell when Fusarium oxysporum is exposed to nicotinaldehyde. We currently investigate in greater details the NAD biosynthesis pathway in Fusarium oxysporum and the mode of action of nicotinaldehyde.

An amazing treasure box: Core effectors in smut fungi and their regulation

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The fungus *U. maydis* causes smut disease in maize. Hallmarks of the disease are large plant tumors developing on all above ground organs of the plant. *U. maydis* is a biotrophic pathogen requiring living plant tissue for colonization. For a successful infection, *U. maydis* needs to suppress plant defense responses and manipulate host physiology for its own benefit. To accomplish this, *U. maydis* secretes a cocktail of several hundred effector proteins. The majority of these proteins lack known protein domains and their function remains to be uncovered. Based on a comparative analysis of six smut genomes we have identified a set of core effectors which are present in all six species. A systematic deletion of the most highly expressed unrelated effector genes in this class resulted in the discovery of four mutants in which virulence was completely abolished. *These mutants* are able to form appressoria that penetrate, but their growth is arrested in epidermal host tissue (*stp* = stop phenotype). A similar phenotype was also observed for mutants lacking the essential effector *pep1* (Doehlemann *et al.,*2009). The observed growth arrest was in all cases accompanied with the elicitation of plant defense responses and plant cell death. Co-IP with individually tagged effectors followed by mass-spectroscopic analysis revealed that four of these completely unrelated effectors form a complex. I will discuss our current efforts to localize the complex, to determine its significance for disease and speculate on its function.

From a comprehensive, time-resolved RNAseq analysis of the biotrophic life cycle of *U. maydis* we identified three modules enriched for effectors. Several transcription factors with high intramodular connectivity to the module associated with tumor formation allowed us to identify one factor which regulates the majority of effector genes in this cluster. Strains in which this transcription factor was deleted, could colonize plants, but tumor formation in leaves was abolished. This suggests that the targets of this transcription factor (termed *nlt1*= no leaf tumors) are involved in tumor formation.

February 26

Session D: Nutritional factors and pathogens of fruits

Chair: Richard A Wilson and Dov Prusky

Anthocyanin and flavonoids role in fruit resistance to fungal pathogens

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Mango fruit of eighty-three cultivars were inoculated with *Colletotrichum gloeosporioides* or stored for two weeks at sub-optimal temperature. Interestingly, red cultivars that accumulate anthocyanin were more resistant to both biotic (anthracnose) and abiotic (chilling) stress. To validate that anthocyanin and red color peel of mango fruit are correlated to biotic and abiotic resistance, red and the green 'Shelly' mango fruit from the exterior and interior of tree canopy of the same trees were evaluated. Red mango fruits accumulated more anthocyanin, flavonoids and antioxidant, while the ripening parameters of both red and green mango fruit were similar. In response to storage at suboptimal temperature, the 'green fruit' responded with accumulation of reactive oxygen species, lipid peroxidation and developed significantly more chilling injury symptoms than the 'red fruit'. Furthermore, 'red fruit' were found to be more resistant to *C. gloeosporioides* inoculation and showed reduction in reactive oxygen species, ethylene, respiration, lipid peroxidation and decay severity both at the red and green side of the red fruit, which suggest the involvement of induced resistance. Interestingly, the resistance of red mango fruit include both induced resistance and direct antifungal activity. Indeed, organic extraction of red fruit peel showed increased inhibition of spore germination and hyphal growth in comparison to green fruit peel. During the characterization of flavonoids and anthocyanins, we found that un-glycosylated flavonoids from mango was more active against pathogenic fungi. To summaries, red mango fruit that accumulate high amount of anthocyanin showed increased resistance to chilling and fungal pathogens by direct antifungal activity and activation of induced resistance. The results point to new agro-technological approaches to extend shelf life and quality of mango fruit.

Host factors modulating *Aspergilus carbonarius* pathogenicity in grapes

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Aspergillus carbonarius, the main cause of severe post-harvest decay of vine fruit, is considered the major source of ochratoxin A (OTA) contamination of grapes and derived products. The factors inducing OTA accumulation by *A. carbonarius* and its contribution to pathogenicity remain unclear. In the present study we analyzed the influence of certain physiological parameters, such as growth substrate and pH, on the expression of OTA biosynthesis genes and on OTA production by *A. carbonarius*. Moreover, the possible role of sucrose, a main carbon source in the substrate, as a regulator of organic acids accumulation and consequent OTA production in vitro has been investigated. The results indicate that high sugar concentrations favor high levels of organic acid production that result in a low final pH, strong induction of the OTA biosynthesis genes, and mycotoxin accumulation. Furthermore, we found that increasing sucrose content had also positive impacts on an expression of the global regulator of secondary metabolites, LaeA. An increased *laeA* expression was observed in high sucrose concentration (15%), which was reduced 7-fold in 0.5% sucrose, suggesting that sugar concentration may play an important role as a regulator of OTA synthesis *in vitro* through induction of *laeA* expression. Deletion of *laeA* in *A. carbonarius* resulted in a drastic decrease in the OTA production and reduction in decay development in grape berries inoculated with *ΔlaeA* deletion mutant compared to the wild-type strain. The results indicate the importance of abiotic factors in LaeA regulation of OTA and other secondary metabolites that contribute to pathogenicity.

Crosstalk between the mitogen activated protein kinase SakA^{HOG1}–MpkC and protein kinase A connects carbohydrate mobilization to cell wall biosynthesis in *Aspergillus fumigatus*

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Invasive aspergillosis is predominantly caused by *Aspergillus fumigatus* and adaptations to stresses experienced within the human host are a prerequisite for the survival and virulence strategies of the pathogen. The central signal transduction pathway operating during hyperosmotic stress is the High Osmolarity Glycerol mitogen-activated protein kinase (MAPK) cascade. *A. fumigatus* SakA and MpkC, orthologues of the *Saccharomyces cerevisiae* Hog1p, constitute the primary regulators of the hyperosmotic stress response. The Δ*sakA* and the double Δ*mpkC* Δ*sakA* mutants were more sensitive to osmotic and oxidative stresses, and to cell wall damaging agents. We compared *A. fumigatus* wild-type transcriptional response (RNAseq) to osmotic stress with the Δ*mpkC*, Δ*sakA*, and Δ*mpkC* Δ*sakA* strains. Our results strongly indicate that MpkC and SakA have independent and collaborative functions during the transcriptional response to transient osmotic stress. Cellular carbohydrate catabolic and trehalose metabolic processes were shown as over represented GO (Gene Ontology) terms among the genes that were down regulated in Δ*sakA* and Δ*mpkC* Δ*sakA* versus wild-type post transfer to 1 M Sorbitol for 10 minutes. Trehalose and glycogen accumulation as well as glucose transport are reduced in these mutants when compared to the wild-type strain upon transient osmotic stress. The Δ*sakA* and Δ*mpkC* Δ*sakA* have reduced protein kinase A (PkcA) activity when exposed to transient osmotic stress. Upon osmotic stress, the Δ*pkcA* mutant has increased trehalose and glycogen while PkcA overexpression reduces this accumulation, indicating PkcA is modulating trehalose and glycogen metabolism. Finally, we demonstrated by co-immunoprecipation that SakA interacts with PkcA upon transient osmotic stress. Our results indicate that there is a crosstalk between MpkC-SakA-PkcA that regulates carbon mobilization upon osmotic and cell wall stresses aiming to strength the cell wall to cope with these different kinds of cellular damage.

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Session E. Concurrent Session: Physiology and Metabolism

Chair: Richard A. Wilson and Dov Prusky

Investigating the biology of plant tissue invasion and cell-to-cell movement by the rice blast fungus *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice production. The fungus is also the causal agent of wheat blast, a disease that now threatens wheat production in South America and South Asia. During plant infection, *M. oryzae* forms a specialised infection structure called an appressorium. The infection cell generates enormous turgor, focused as mechanical force to breach the rice cuticle. Re-polarisation of the appressorium requires a hetero-oligomeric septin complex that organises a toroidal F-actin network at the base of the appressorium. This allows the fungus to invade epidermal cells and develop biotrophic invasive hyphae. Septin-mediated plant infection is controlled by NADPH oxidase activity and a regulated burst of reactive oxygen species occurs within the appressorium. The process is regulated by a turgor sensing protein kinase, which can sense when optimal appressorium turgor is achieved and the switch to polarised growth is triggered. A pressure-mediated cell cycle checkpoint is also necessary for initiation of septin activation and the re-orientation of the cortical F-actin cytoskeleton. Once tissue is invaded the fungus undergoes differential expression and secretion of a large repertoire of effector proteins that are delivered to the apoplastic space which surrounds invasive hyphae, or directed into plant cells. The fungus also undergoes distinct physiological changes, including activation of enzymes associated with utilisation of a broad spectrum of carbon sources, as we as distinct secondary metabolic pathwys. *M. oryzae* suppresses plasmodesmatal immunity in order to facilitate its spread from cell-to-cell in plant tissue. This is controlled by a specific MAP kinase signalling pathway and requires septin-dependent hyphal constriction to enable the fungus to spread rapidly in rice tissue.

Evolution of host range is associated with carbohydrate and protein metabolism in *Colletotrichum* spp.

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Colletotrichum spp. cause anthracnose disease on a wide range of agronomically important plant species and exhibit a broad diversity of host range, host specificity and reproductive behaviors. We leveraged the growing number of genome sequences available for *Colletotrichum* spp. and performed a comparative analysis of gene content to find associations with host range and host specificity. The predicted protein sequences from each species were classified into protein families using a variety of tools. Hierarchical clustering of gene family and functional domain assignments, and phylogenetic analyses revealed lineage specific losses of secreted carbohydrate-active enzymes (CAZymes) and protease encoding genes in species that have narrow host range as well as expansions of these families in the acutatum and gloeosporioides species complexes. Members of these species complexes are broad host range pathogens, suggesting that the higher number in CAZy and protease diversity may be associated with the ability to infect multiple host species. This result highlights the similarity in both secretomes and whole proteomes of these species complexes and suggests that their gene family content, especially their repertoires of CAZymes and peptidases are the product of recent, lineage specific expansions of these families independently in each species complex. Interestingly, phylogenetic analyses of the CAZyme and peptidase families revealed that, in contrast to our expectations, gene loss in other *Colletotrichum* species is as important, if not more important force driving the evolution of gene family size. These results are consistent with the idea that different lifestyles, hosts and host tissues present different types of carbohydrate substrates to the pathogen this is reflected by each species' CAZyme and peptidase repertoire.

Functional distinction of hyphal compartments

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Hyphae grow of higher fungi grow at their apex and are compartmentalized by septa that have a central pore enabling cytoplasmic streaming. Peroxisome-derived Woronin bodies however can plug these pores. Incidence of plugging increases in time switching the unicellular organization of young hyphal compartments into a multicellular one in older compartments. It was assessed whether the multicellular organization contributes to apical growth of hyphae and how growth is affected when Woronin bodies are absent. Hyphae of the wildtype strain and a Δ*hexA* strain that lacks Woronin bodies had a similar morphology and growth rate. A total of 58% and 17% of the hyphae continued growing, respectively, after dissecting the 2nd compartment. Growth rate of these hyphae was not affected, even when the carbon or nitrogen source was limiting. Dissection at a fixed position of 400 µm from the apex revealed that all wild-type and Δ*hexA* hyphae stopped growing when the first septum was positioned > 400 µm from the apex, while 81 % and 57% of the hyphae, respectively, continued growing when the first septum located < 400 µm from the tip. When apical compartments were dissected, normal growth from subapical compartments was recovered in wild type hyphae but not in Δ*hexA* hyphae. Together, we showed for the first time that apical compartments are self-sustaining in growth

Foliar sugar accumulation enhances priming of the salicylic acid-mediated defense response

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We have investigated the role of carbohydrate partitioning and allocation in Arabidopsis source leaves in the compatible interaction with the fungal hemibiotroph *Colletotrichum higginsianum*, which exhibits an initial biotrophic and an ensuing necrotrophic colonization phase.

Arabidopsis mutants with impaired starch turnover are more susceptible towards *C. higginsianum* infection and a strong negative correlation between diurnal carbohydrate accumulation and fungal proliferation is evident in the investigated mutants. Our results demonstrate that mutants suffering from nocturnal carbon shortage show a dampened salicylic acid (SA) response that impairs defense especially during the necrotrophic colonization phase.

On the other hand, Arabidopsis double mutants lacking the sucrose transporters SWEET11 and SWEET12 show constantly elevated carbohydrate levels and are more resistant towards *C. higginsianum*. Analysis of YFP reporter plants as well as single and double mutants suggests that a lack of these transporters does not affect pathogen nutrition during the initial biotrophic phase. Instead, our data identify enhanced priming of the SA pathway in *sweet11/sweet12* double mutant as the cause of increased resistance

Branched chain amino acid biosynthesis genes and regulators in *Aspergillus nidulans*

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The branched chain amino acids (BCAA) leucine, isoleucine, and valine are important precursors for biosynthesis of proteins and secondary metabolites. The BCAA biosynthesis pathway is well characterized in *Saccharomyces cerevisiae*. However, recent work on BCAA pathway enzymes in *Aspergillus* revealed differences in the number of genes for several steps. The genes for the final two steps of leucine biosynthesis, catalyzed by β-isopropylmalate dehydrogenase (β-IDH) and BCAA aminotransferase (BAT), have not yet been characterized in the Aspergilli. The BATs also catalyze the final step of isoleucine and valine production. In *S. cerevisiae*, there is one β-IDH gene and two BAT genes. Using protein sequence similarity we identified two β-IDH genes in *A. nidulans*, *leuD* and *leuE.* We show that deletion of *leuD*, but not *leuE*, causes leaky leucine auxotrophy. The *leuD*Δ *leuE*Δ double mutant is a strict leucine auxotroph indicating that both genes encode functional enzymes. Quantitative RT-PCR reveals that *leuE* up-regulation compensates for loss of *leuD*. We identified, using protein sequence similarity, six *A. nidulans* BAT genes, *batA-F*. Deletion of these six genes separately does not confer BCAA auxotrophy. However, the double deletion mutant lacking the two most highly expressed BAT genes is a BCAA auxotroph, suggesting that these two genes encode the predominant biosynthetic enzymes and the other *BAT genes* may have evolved new roles. Two of the other BAT genes lie in the aspercryptins secondary metabolism gene cluster and likely catalyze biosynthesis of unusual BCAA components of aspercryptins. We have characterized the regulation of leucine biosynthesis pathway genes by the transcription factor LeuB. The *leuB*Δ mutant is a leaky leucine auxotroph. We have identified a LeuB paralog, LeuR, by sequence similarity. Deletion of *leuR* does not confer leucine auxotrophy. However, the *leuB*Δ *leuR*Δ double mutant is a strict leucine auxotroph, indicating that LeuR also regulates leucine biosynthesis.

A taste for ‘sour’ sugars: characterization of a highly efficient D-galacturonic acid metabolism in the basidiomycete yeast genus *Rhodosporidium*

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Saccharides released during the degradation of lignocellulosic material (e.g. for biorefinery purposes) are traditionally fermented using strains of *Saccharomyces cerevisiae*, which however has only a very limited ability to metabolize all available sugars. Thus, major efforts have to be undertaken to engineer catabolic pathways for sugars such as D-xylose, L-arabinose and D-galacturonic acid (GalA) in this ascomycete yeast. Still, the metabolism of some of these monosaccharides remains slow. Better catabolic pathways have to be transferred from fungal strains with a high ability to utilize these monosaccharides.

The oleaginous basidiomycete yeasts Rhodosporidium *toruloides* and *Rhodotorula mucilaginosa*were discovered as saprophytes colonizing for example pectin-rich olives and wine grapes and are known to be able to produce large quantities of carotenoids in short time. However, they also thrive on GalA, the main component of the pectin backbone. This is remarkable, since GalA is an oxidized sugar and therefore energetically difficult to catabolize by fungi. In addition, the molecular mechanisms behind the pectinolytic capabilities of basidiomycete fungi are almost entirely unknown.

In this work, we characterized the GalA metabolism pathway of these special yeasts on multiple levels. Following physiological growth assays with multiple strains, RNAseq analyses were performed in *R. toruloides* to identify genes that are differentially expressed on GalA and pectin. Moreover, RB-TDNAseq, a recently reported novel method for fitness scoring in barcoded TDNA-transformed populations, was used to identify targets affecting fitness during growth on GalA. This way, we identified the enzymes being involved in the GalA catabolism, which were heterologously expressed and their kinetics determined. Moreover, sugar transporters and a novel transcription factor putatively regulating the GalA metabolism were identified. Taken together, our results demonstrate that the genes from *R. toruloides* and *R. mucilaginosa* are promising candidates for rational engineering of an efficient GalA metabolism for example in *S. cerevisiae*.