36th New Phytologist Symposium

Cell biology at the plant–microbe interface

29 November – 1 December 2015
Eden Hotel Wolff, Munich, Germany

New Phytologist

Programme, abstracts and participants

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Scientific Organizing Committee

Silke Robatzek *(The Sainsbury Laboratory, Norwich, United Kingdom)*
Peter Dodds *(CSIRO Agriculture, Canberra, Australia)*
Maria Harrison (*Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY, USA)*

New Phytologist Organization

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Programme, abstracts and participant list compiled by Jill Brooke
'Cell biology at the plant–microbe interface' logo by A.P.P.S., Lancaster, United Kingdom

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Information for Delegates

Symposium location
The 36th New Phytologist Symposium will be held at the Eden Hotel Wolff, Arnulfstraße 4, 80335 Munich, Germany. Telephone: (089) 55 11 5-0.
All presentations will be given in the Edensaal.

Directions and a map can be found at the back of this abstract book. More maps and driving instructions can be found at http://www.eden-hotel-wolff.de/english/hotel-location/

Catering
Coffee breaks and the poster session will be held in the Europasaal. During the poster session on Sunday evening there will be a finger buffet with beer, wine and soft drinks available.
Lunch will be served in the Peter & Wolff hotel restaurant.
The symposium dinner will be held at the Hofbräuhaus on Monday evening. The dinner is for all speakers and delegates and is included in your registration fee. The map at the back of this abstract book shows the location of the Hofbräuhaus in relation to the Eden Hotel Wolff. We plan to walk from the Eden Hotel Wolff (approx. 20 min), so please meet in the hotel reception at 18:30. If you prefer to go by taxi these are available opposite the hotel.

Accommodation
If you are staying at the Eden Hotel Wolff please note that check-in is from 14:00 and you should check-out before 12:00.

Posters
Posters should be prepared so that they are no larger than A0 size, portrait orientation (118 cm high x 84 cm wide). Posters should be put up during registration (09:00–10:30 on 29th November) and will be displayed for the duration of the meeting. Delegates are welcome to view posters during coffee and lunch breaks, but there will be a dedicated poster session at 16:45–19:30 on Sunday 29 November. Please stand by your poster for part of this session (we appreciate as poster presenters you will also want to view and discuss the other posters). Please note there will be prizes for the best poster presentations.

Internet access
Free wifi will be provided throughout the venue. If you are staying at the hotel wifi access information will be provided when you check in. Please ask a member of the *New Phytologist* team for access if you are not staying at the Eden Hotel Wolff and they will be happy to help.

Social media
We encourage all attendees to join in discussions on social media sites. Follow @NewPhyt on Twitter and fb.com/NewPhytologist on Facebook for updates before, during and after the meeting. Please use the hashtag #36NPS in all of your tweets.

Contact
For further information, and in case of any emergencies, please contact Helen Pinfield-Wells. Email: h.pinfield-wells@lancaster.ac.uk, np-symposia@lancaster.ac.uk; tel: +44 7966 450 389.

Meeting Programme

Sunday 29 November

09:00–10:30	Registration
10:30–10:45	Welcome, Introductions and Information
Session 1: Invasion and spreading strategies **Chair: Maria Harrison**	
10:45–11:25	**S1-1** Ton Bisseling The evolutionary relation between AM fungal and rhizobium endosymbiosis
11:25–12:05	**S1-2** Andrea Genre This way in – recognition and accommodation of arbuscular mycorrhizal fungi by their host plants
12:05–12:25	Selected poster abstract talk 1 – Tolga Bozkurt **P6** An effector from Irish potato famine pathogen mediates selective autophagic cargo sorting
12:25–13:30	**Lunch – Peter & Wolff Restaurant (Hotel Restaurant)**
13:30–14:10	**S1-3** Richard O'Connell Hemibiotrophic interfaces and invasion strategies of *Colletotrichum* fungi
14:10–14:50	**S1-4** Nick Talbot Investigating the cell biology of appressorium-mediated plant infection and tissue invasion by the rice blast fungus *Magnaporthe oryzae*
14:50–15:10	Selected poster abstract talk 2 – Joëlle Fournier **P17** Infection chamber remodeling allows rhizobial entry into *Medicago truncatula* root hairs
15:10–15:45	**Break**
15:45–16:45	**Keynote lecture** Karin Schumacher Live and in colour: improved tools for multi-parameter imaging
16:45–17:15	Flash talks
17:15–19:30	**Poster session with buffet and drinks – Europasaal**

Monday 30 November

Session 2: Accommodation of specialized microbial structures **Chair: Silke Robatzek**	
09:00–09:40	**S2-1** Maria Harrison

	Development of arbuscules and the periarbuscular membrane during AM symbiosis
09:40–10:20	**S2-2** Sophien Kamoun Membrane trafficking at the haustorial interface
10:20–10:40	Selected poster abstract talk 3 – Rik Huisman **P27** Specialization of exocytosis pathways to maintain a stable symbiotic host–microbe interface
10:40–11:10	**Break**
11:10–11:50	**S2-3** Noriko Inada Pathogenic modulation of plant-specific RAB GTPase-mediated host membrane trafficking at the interface between plants and obligate biotrophic pathogens
11:50–12:30	**S2-4** Martin Parniske Symbiosis-related genes sustain the development of a downy mildew pathogen on *Arabidopsis thaliana*
12.30–12:50	Selected poster abstract talk 4 – Andreas Keymer **P30** The development of hyphal symbionts and pathogens relies on fatty acid biosynthesis by the plant host
12:50–13:50	**Lunch - Peter & Wolff Restaurant (Hotel Restaurant)**
Session 3: Dynamic localization of receptors **Chair: Peter Dodds**	
13:50–14:30	**S3-1** Jane Parker Connecting TNL receptors to the transcriptional defense network
14:30–15:10	**S3-2** Christine Faulkner Specialisation of pathogen perception and immune signalling at plasmodesmata
15:10–15:30	Selected poster abstract talk 5 – Kyaw Aung **P2** A bacterial effector targets host plasmodesmata to promote pathogen virulence in plants
15:30–16:00	**Break**
16:00–16:40	**S3-3** Silke Robatzek Transport-regulated immunity
16:40–17:20	**S3-4** Shunyuan Xiao Towards understanding extra-haustorial membrane-oriented protein targeting and host defense at this host-pathogen interface
17:20–17:40	Selected poster abstract talk 6 – Corinna Hofer **P25**

	Identification of a novel receptor-like kinase that is associated with membrane micro-domains and regulates plant immunity
19:00	**Symposium Dinner at Hofbräuhaus Munich**

Tuesday 01 December

Session 4: Delivery and function of microbial molecules
Chair: Ton Bisseling

09:00–09:40	**S4-1** Peter Dodds Identifying rust effectors and their roles in disease and immunity
09:40–10:20	**S4-2** Regine Kahmann The secreted effector repertoire of smut fungi and finding out where they function
10:20–10:40	Selected poster abstract talk 7 – Philip Albers **P1** HopZ1a targets a remorin implicated in membrane-associated defence signalling
10:40–11:10	**Break**
11:10–11:50	**S4-3** Claire Veneault-Fourrey JAZ proteins in poplar roots: a checkpoint for establishment of mutualistic ectomycorrhizal interactions?
11:50–12:30	**S4-4** Barbara Valent Effector delivery by the blast fungus during biotrophic invasion of rice
12:30–12:50	Selected poster abstract talk 8 – Libera Lo Presti **P36** An assay for entry of pathogen effectors into host cells?
12:50–13:50	**Lunch - Peter & Wolff Restaurant (Hotel Restaurant)**

Session 5: Cell surface response
Chair: Sophien Kamoun

13:50–14:30	**S5-1** Hans Thordal-Christensen Membrane trafficking in plant cells attacked by powdery mildew fungi
14:30–15:10	**S5-2** William Underwood Forward genetic screening reveals new insights into cellular processes governing local recruitment of defenses to the plant–microbe interface
15:10–15:30	Selected poster abstract talk 9 – R. Thomas Nakano **P40** Transcriptional co-regulation between ER bodies and indole glucosinolate metabolism, a potential strategy to maximize the efficiency of defensive traits
15:30–16:00	**Break**

16:00–16:20	Selected poster abstract talk 10 – Hannah Kuhn **P32** mlo-mediated powdery mildew resistance of Arabidopsis coincides with differentially altered MAMP-triggered responses
16:20–17:00	**S5-3** Gunther Döhlemann Virulence strategies of the fungal biotrophs: lessons from the *Ustilago maydis*–maize model system
17:00–17:40	**S5-4** Thomas Ott The formation of an infection-related membrane domain is controlled by the sequential recruitment of scaffold and receptor proteins
17:40–18:00	**Closing remarks**
18:00	**End**

Speaker Abstracts

S=speaker abstract; P=poster abstract

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Speaker Abstracts

Session 1: Invasion and spreading strategies
Chair: Maria Harrison

The evolutionary relation between AM fungal and rhizobium endosymbiosis **S1.1**

TON BISSELING **10:45–11:25**

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The AM fungal and rhizobium endosymbiosis have in common that the microsymbionts are accommodated intracellularly, in specialized membrane compartments that form a symbiotic interface. Both symbiotic interactions are set in motion by a shared common signalling pathway that is activated by specific lipochito-oligosaccharides, the Nod and Myc factors. Legumes can establish both symbiotic interactions although Nod and Myc factors activate the common symbiotic signalling pathway. They are perceived by different receptors. The legume Nod factor receptor most likely evolved from the Myc receptor by gene duplication and subsequent neo-functionalization. This hypothesis is based on studies on Parasponia. The Parasponia rhizobium symbiosis evolved far more recent than the legume rhizobium symbiosis. Further, the Parasponia Nod factor receptor has maintained its ancestral function as it is also a Myc factor receptor. The formation of the symbiotic interface, in both endosymbiotic interactions, is triggered by Nod/Myc factor signalling.
The formation of the symbiotic interfaces involves a specific exocytosis pathway characterised by specific v-SNAREs (VAMP72). Also, a symbiosis specific t-SNARE (SYP13II) is a key component of this ancient exocytosis pathway that is dedicated to endosymbiosis. In most dicots *SYP13II* is alternatively spliced resulting in two isoforms, named SYP13IIα and SYP13Iiβ, of which SYP13Iiα is the ancestral form. By specific knockdown of the Medicago SYP13IIα (*MtSYP132α*), we show that this isoform is essential for the formation of a stable symbiotic interface in both endosymbioses. So the rhizobium nodule symbiosis co-opted both the signalling, as well as the cellular mechanism from the AM fungal symbiosis.
A comparison with a biotrophic pathogenic interaction (Medicago – *Phytophthora palmivora*) showed that neither the signalling nor cellular mechanisms are shared with the endosymbiotic interactions.

This way in – recognition and accommodation of arbuscular mycorrhizal Fungi by their host plants

S1.2

ANDREA GENRE, PAOLA BONFANTE

11:25–12:05

andrea.genre@unito.it

Department of Life Sciences and Systems Biology, University of Turin, Viale Mattioli 25, Torino, 10125, Italy

Arbuscular mycorrhizas (AM) are symbiotic associations involving up to 80% of terrestrial plants and symbiotic fungi belonging to Glomeromycota. By colonizing the root through the development of inter- and intracellular hyphae and the formation of the highly branched structures, called arbuscules, AM fungi provide many benefits to their hosts, among which the enhancement of plant mineral nutriiton is the most widely acknowledged. The presence of a symbiotic interface compartment around intracellular fungal structures is a hallmark of the biotrophic condition of AM fungi, since it enables fungal development inside the plant cell space, while preserving its integrity.
The presentation focuses on the plant perception of AM fungi and the events associated to their accommodation within the host cell. Our results, largely based on in vivo confocal microscopy, and supported by transcriptomic analyses demonstrate that the process of interface construction initiates with the recognition of fungal bioactive molecules and adhesion of a hyphopodium to the root epidermis. Epidermal cells respond with repetitive oscillations (spiking) in nuclear calcium concentration, which are a central element in the signaling pathway that controls both AM and symbiotic nitrogen fization. The activation of this signaling pathway leads to the assembly of the prepenetration apparatus (PPA), a columnar cytoplasmic aggregation. By taking advantage of a range of fluorescent protein markers we show that the host plasma membrane proliferates within the PPA, in an extensive exocytotic process, leading to the assembly of the perifungal membrane and symbiotic interface, in advance of hyphal tip growth. The involvement of cell division-related events during root colonization adds novel parallels between AM and symbiotic nitrogen fixation.

Hemibiotrophic interfaces and invasion strategies of *Colletotrichum* fungi

S1.3

RICHARD O'CONNELL[1], GUILLAUME ROBIN[1], JEAN-FÉLIX DALLERY[1], SANDRINE PIGNÉ[1], ULLA NEUMANN[2]

13:30–14:10

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[1]UMR1290 BIOGER, INRA-AgroParisTech, Thiverval-Grignon, France; [2]Max Planck Institute for Plant Breeding Research, Köln, Germany

Colletotrichum fungi cause anthracnose and blights on many major crop plants worldwide, and the *C. higginsianum–Arabidopsis* provides a model for molecular dissection of these devastating diseases. *C. higginsianum* has a hemibiotrophic infection strategy involving differentiation of a series of cell-types adapted for direct penetration of epidermal cells (melanized appressoria), growth inside living plant cells (bulbous biotrophic hyphae) and tissue destruction (filamentous necrotrophic hyphae). During infection these fungal structures develop specialized interfaces with plant cells. Thus, within the 200 nm-diameter penetration pore the appressorial plasma membrane makes direct contact with the plant cuticle, providing a nanoscale 'window' for sensing plant signals and focal delivery of fungal effectors. Biotrophic hyphae are tightly enveloped by an expanded host plasma membrane, which develops a specialized composition through the exclusion of some host proteins and specific targeting of others. Novel compartments called 'interfacial bodies' formed on the surface of biotrophic hyphae act as reservoirs for effector accumulation.

Genome-wide expression profiling revealed waves of gene activation linked to each hemibiotrophic stage transition. For example, although appressoria formed *in vitro* resemble those *in planta*, comparison of their transcriptomes showed >1,500 genes are induced upon host contact, which encode a large array of effectors, secondary metabolism enzymes, membrane transporters and carbohydrate-active enzymes. This suggests that plant signals sensed by appressoria reprogram fungal gene expression in preparation for host invasion. Progress towards understanding how this coordinated expression of pathogenicity-related genes is orchestrated will be presented.

Investigating the cell biology of appressorium-mediated plant infection and tissue invasion by the rice blast fungus *Magnaporthe oryzae*

S1.4

NICHOLAS J. TALBOT, LAUREN S. RYDER, YOGESH K. GUPTA, XIA YAN, GEORGE R. LITTLEJOHN, MIRIAM OSES-RUIZ, WASIN SAKULKOO, DARREN M. SOANES, YASIN F. DAGDAS, TOM MENTLAK, MICHAEL J. KERSHAW

14:10–14:50

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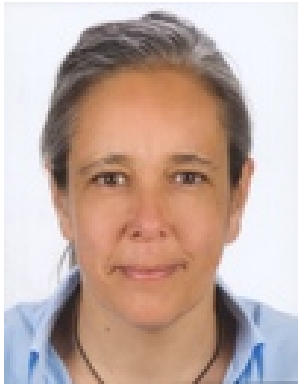
School of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter EX4 4QD, United Kingdom

Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice production. During plant infection, *M. oryzae* forms a specialised infection structure called an appressorium. The infection cell generates enormous turgor, which is focused as mechanical force to breach the rice cuticle and facilitate entry of the fungus into plant tissue. We have observed that a single round of mitosis occurs prior to appressorium morphogenesis and precedes autophagic cell death of the three-celled conidium, which is necessary for plant infection. An S-phase checkpoint is necessary for initiation of appressorium development and maturation of the appressorium requires the G2-M transition to have occurred. Furthermore, penetration peg emergence from the appressorium also requires an S-phase checkpoint to be traversed. Re-polarisation of the appressorium requires a hetero-oligomeric septin GTPase complex required for re-modelling a toroidal F-actin network at the base of the appressorium. This allows the host cuticle to be breached and leads to invasion of epidermal cells by the biotrophic invasive hyphae of *M. oryzae*. Septin-mediated plant infection is controlled by NADPH oxidase activity and a regulated burst of reactive oxygen species. A specialised Nox2 NADPH oxidase-tetraspanin complex is necessary for septin-mediated control of actin dynamics. The appressorium pore is also the site of polarised exocytosis during plant infection and the octameric exocyst complex localises to the pore in a septin-dependent manner. Once tissue is invaded the fungus undergoes differential expression and secretion of a large repertoire of effector proteins destined either for the apoplastic space which surrounds invasive hyphae, or directed instead into plant cells. How host cell delivery is achieved is not known, but it appears to involve a specialised structure known as the biotrophic interfacial complex (BIC), a plant membrane-rich body where effectors accumulate. Progress in understanding the cell biology of cuticle rupture, tissue invasion and cell-to-cell movement will be presented.



Live and in colour: improved tools for multi-parameter imaging

Keynote Lecture

15:45–16:45

MELANIE KREBS, RAINER WAADT, STEFAN SCHOLL, KARIN SCHUMACHER

karin.schumacher@cos.uni-heidelberg.de

Plant Developmental Biology, Centre for Organismal Studies Heidelberg, Im Neuenheimer Feld 230, Heidelberg, 69120, Germany

Dynamic changes in $[Ca^{2+}]_{cyt}$ and pH are integral components of many signalling pathways including those taking place at the plant–microbe interface. My lab studies the transport and trafficking systems that control cellular pH and Ca^{2+}-homeostasis and we are particularly interested in how the conserved molecular machinery is adapted to the unique architecture of plant cells. *In vivo* imaging using improved genetically-encoded sensors now allows us to study the spatio-temporal dynamics of pH and $[Ca^{2+}]_{cyt}$ at subcellular resolution. In my presentation I will thus not only cover our recent work on pH-regulation in the plant endomembrane system but will report on our attempts to establish material and protocols for simultaneous measurement of key parameters including pH, $[Ca^{2+}]_{cyt}$ and ROS.

Session 2: Accommodation of specialized microbial structures
Chair: Regine Kahmann

Development of arbuscules and the periarbuscular membrane during AM symbiosis **S2.1**

MARIA J. HARRISON, XINCHUN ZHANG, SERGEY IVANOV, HEE-JIN PARK, DANIELA FLOSS

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

09:00–09:40

Most vascular flowering plants have the ability to form endosymbioses with arbuscular mycorrhizal (AM) fungi via which the plant enhances its access to mineral nutrients while the fungal endosymbiont acquires carbon from the plant. Development of AM symbiosis involves the differentiation of both symbionts to create the symbiotic state. Following entry into the root cortical cells, terminal differentiation of the fungus, coordinated with reorganization of the plant cell, enables development of a highly branched hypha called an arbuscule housed within a new membrane-bound compartment within the cortical cell. The periarbuscular membrane closely follows the contours of the arbuscule and two large domains, the 'branch' and 'trunk' domains can be defined by their physical locations and also by their resident proteins; a phosphate transporter, MtPT4 and two half-ABC transporters, STR and STR2, are present exclusively in the branch domain. Differentiation of the arbuscule, the transcriptional induction of the transporter genes and changes in direction of secretion within the cell are tightly coordinated and this results in the polarized deposition of the transporters exclusively in branch domain of the periarbuscular membrane. To determine the mechanisms underlying these events and how they are coordinated, we are investigating transcriptional regulation of periarbuscular membrane-resident protein genes and also the proteins involved in deposition of the periarbuscular membrane. An EXO70 protein that is present exclusively in plants forming AM symbiosis is one of the proteins essential for this process. *Medicago truncatula exo70i* mutants are unable to support arbuscule development and show limited incorporation of STR and STR2, into the periarbuscular membrane. EXO70I interacts with Vapyrin, a protein of unknown function that is essential for arbuscule development. The role of EXO70I in arbuscule development will be discussed.

Membrane trafficking at the haustorial interface

S2.2

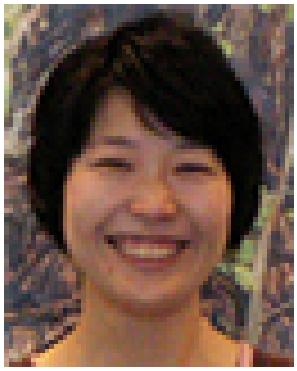
SOPHIEN KAMOUN[1], YASIN F. DAGDAS[1], KHAOULA BELHAJ[1], BENJAMIN PETRE[1], JOE WIN[1], TOLGA O. BOZKURT[2]

09:40–10:20

Sophien.Kamoun@sainsbury-laboratory.ac.uk

[1]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, United Kingdom; [2]Imperial College London, Department of Life Sciences, London, United Kingdom

Many plant pathogenic and symbiotic microbes produce specialized structures that invade host cells but remain enveloped by host-derived membranes. The mechanisms underlying the biogenesis and functions of such host–microbe interfaces are poorly understood. The Irish potato famine pathogen *Phytophthora infestans* is an oomycete hemibiotroph that infects solanaceous plants. During its biotrophic phase, *P. infestans* forms haustoria. A host-derived membrane, called the extrahaustorial membrane (EHM), separates haustoria from the plant cell and constitutes the haustorial interface. Some *P. infestans* strains infect *Nicotiana benthamiana* and develop haustoria in infected leaf cells. We have exploited the *N. benthamiana* experimental system to perform fast-forward cell biology of the haustorial interface. This revealed dynamic changes in host membrane compartment formation and rerouting in haustoriated cells. In particular, we discovered that selective autophagy and other trafficking pathways are diverted to the pathogen interface. Our working model is that *P. infestans* RXLR effectors co-opt host membrane trafficking to promote host colonization.



Pathogenic modulation of plant-specific RAB GTPase-mediated host membrane trafficking at the interface between plants and obligate biotrophic pathogens

S2.3

NORIKO INADA[1], SHIGEYUKI BETSUYAKU[1,2], TAKASHI L. SHIMADA[1], KAZUO EBINE[1], EMI ITO[1], NATSUMARO KUTSUNA[3], SEIICHIRO HASEZAWA[3], YOSHITAKA TAKANO[4], HIROO FUKUDA[1], AKIHIKO NAKANO[1,5], TAKASHI UEDA[1,2]

11:10–11:50

noriko@bs.s.u-tokyo.ac.jp

[1]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Bunkyo-ku, Tokyo, Japan; [2]Japan Science and Technology Agency (JST), PRESTO, Honcho Kawaguchi, Saitama, Japan; [3]Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwanoha, Kashiwa, Chiba, Japan; [4]Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan; [5]Live Cell Super-resolution Imaging Research Team, RIKEN Center for Advanced Photonics, Hirosawa, Wako, Saitama, Japan

ARA6 is a plant-specific type of RAB5 GTPase that plays a role in endosomal trafficking, which is distinct from the trafficking event regulated by canonical RAB5 GTPases. We found that ARA6 is localized at the interface between the host plant and biotrophic fungal and oomycete pathogens, and that pathogens likely modulate ARA6 to successfully establish infection. Biotrophic fungi and oomycetes colonize living plant tissues by establishing specialized infection hyphae, the haustorium, within host plant cells. We found that *Arabidopsis thaliana* ARA6 is localized to the specialized membrane that surrounds the haustorium, the extrahaustorial membrane (EHM), formed by the *A. thaliana*-adapted powdery mildew fungus *Golovinomyces orontii*. Whereas the conventional RAB5 GTPase ARA7 was also localized to the EHM, endosomal SNARE and RAB5-activating proteins were not, which indicates that the EHM has modified endosomal characteristics. The overexpression of constitutively active ARA6, but not constitutively active ARA7 or wild type ARA6, impaired the full proliferation of the fungus, thus indicating the importance of ARA6 in plant–fungal interactions. The recruitment of host ARA6 to the EHM was shared by the barley-adapted powdery mildew fungus *Blumeria graminis* f.sp. *hordei* and oomycete *Hyaloperonospora arabidopsidis*, but the extrahyphal membrane surrounding the hypha of the hemibiotrophic fungus *Colletotrichum higginsianum* at the biotrophic stage was devoid of ARA6. Our discovery sheds light on a novel function of plant-specific ARA6 as well as the mechanism of biotrophic pathogen infection.

Symbiosis-related genes sustain the development of a downy mildew pathogen on *Arabidopsis thaliana*

S2.4

ALINE BANHARA[1*], MARTINA K. RIED[1*], ANDREAS BINDER[1], ANDREA A. GUST[2], CAROLINE HÖFLE[3], RALPH HÜCKELHOVEN[3], THORSTEN NÜRNBERGER[2], MARTIN PARNISKE[1]

11:50–12:30

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*[1] University of Munich (LMU), Faculty of Biology, Genetics, Großhaderner Str. 4, 82152 Martinsried, Germany; [2]University of Tübingen, Center for Plant Molecular Biology, Plant Biochemistry, Auf der Morgenstelle 32, 72076 Tübingen, Germany; [3]Technische Universität München, Chair of Phytopathology, Center of Life and Food Sciences Weihenstephan, Emil-Ramann-Str. 2, 85350, Freising-Weihenstephan, Germany. *Authors contributed equally*

An ancient genetic program for intracellular infection of plant roots by symbiotic arbuscular mycorrhizal (AM) fungi is conserved among angiosperms. *Arabidopsis* accommodates haustoria of the oomycete *Hyaloperonospora arabidopsidis*, intracellular feeding organs structurally similar to fungal arbuscules. We observed that, without constitutive resistance or exacerbated defence activation, *H. arabidopsidis* produces less sporangiophores and more morphologically altered haustoria on *Arabidopsis* mutants defective in homologs of genes required for plant root symbiosis. These findings reveal genetic commonalities between the host plant's programs for the development of intracellular accommodation structures in symbiosis and disease. While such exploitation of symbiotic programs by pathogens might explain the consistent deletion of symbiosis genes from five independent plant lineages after the loss of AM symbiosis, it raises the question which evolutionary drives retained the symbiosis core gene set in an otherwise AM-asymbiotic plant?

Session 3: Dynamic localization of receptors
Chair: Peter Dodds

Connecting TNL receptors to the transcriptional defense network **S3.1**

JANE E. PARKER[1], HAITAO CUI[1], JINGDE QIU[1], DEEPAK BHANDARI[1], CLEMENTINE LE ROUX[1,2], DMITRY LAPIN[1], THOMAS GRIEBEL[1], DOMINIQUE TREMOUSAYGUE[2], LAURENT DESLANDES[2]

13:50–14:30

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Intracellular pathogen recognition in plants is mediated by a large, polymorphic family of NLR receptors. NLRs become activated by pathogen effector interference with basal defense mechanisms in different parts of the cell and this is transduced to cytoplasmic and/or nuclear resistance pathways. Ultimately, signaling in effector-triggered immunity (ETI) converges on the nuclear transcriptional machinery to reprogram cellular defense networks. Some NLRs are activated in the cytoplasm and others in the nucleus. Some localize dynamically between cell compartments. A number of nucleocytoplasmic NLR receptors function inside nuclei by directly modulating transcription factor (TF) activities. Nevertheless, molecular events between NLR activation and regulation of defense pathways are poorly understood. Also, it is unclear how signals from NLRs activated in distal parts of the cells converge on a rather conserved transcriptional system. We are interested in events connecting the bacterial effector activation of an Arabidopsis nuclear NLR receptor pair, RRS1 and RPS4, to cellular reprogramming. RRS1/RPS4 become activated at the chromatin via an RRS1-integrated WRKY TF decoy domain to switch effector disabling of multiple WRKY TFs during immunity suppression into ETI. RRS1/RPS4 belong to the TNL receptor sub-class and, like other characterized TNLs, interact with and signal through the basal immunity regulator, EDS1. EDS1 operates with two direct partners, PAD4 and SAG101, and a protein structure analysis is providing some important leads to how EDS1 heterodimers work in basal and TNL resistance. Together with Laurent Deslandes and colleagues at LIPM Toulouse, we are working to characterize functional interactions between RRS1/RPS4, the RRS1/RPS4-recognized effectors, and EDS1 with its partners, and how these relate to receptor molecular associations at the DNA. Our aim is to connect NLR complex activation with steering of the defense network in pathogen resistance.

Specialisation of pathogen perception and immune signalling at plasmodesmata

S3.2

CÉCILIA CHEVAL, CHRISTINE FAULKNER

14:30–15.10

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Multicellularity depends upon the co-ordination of responses between cells and tissues. In plants, defence responses require the regulation of intercellular communication and this is mediated by plasmodesmata, membrane-lined pores that connect neighbouring cells. Plasmodesmata close and restrict intercellular molecular flux in response to the perception of a number of different pathogen-associated molecular patterns (PAMPs). For chitin, this response is mediated by the LysM receptor protein LYM2 which is located at plasmodesmata. Significantly, this response is independent of chitin perception and signalling via the well-described chitin receptor kinase CERK1, indicating that the plasmodesmal membrane contains specialised signalling domains. It is well established that LysM receptor proteins and receptor kinases form complexes for the perception of ligands. Intercellular flux assays indicate that two additional LysM receptor kinases are required for chitin-triggered plasmodesmal closure, supporting the hypothesis that a specialised complex mediates plasmodesmal signalling. Both calcium and reactive oxygen species signalling act downstream of CERK1 mediated chitin perception and can trigger plasmodesmal responses. We are investigating their role in PAMP-triggered PD closure. Gene expression analysis indicates that chitin-triggered plasmodesmal closure positively regulates salicylic acid (SA) defence signalling, raising the possibility that SA distribution is controlled by the dynamics of the symplast. Thus, our data establishes that plasmodesmal receptors trigger changes to intercellular communication that regulate the initiation and co-ordination of defence responses.

Transport-regulated immunity

S3.3

SARA BEN KHALED, GILDAS BOURDAIS, MICHAELA KOPISCHKE, JELLE POSTMA, SILKE ROBATZEK

16:00–16.40

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Subecellular transport processes regulate the localization of key components of the plant's immune system. Our main research focus has been how pattern recognition receptors (PRRs), the primary sensors of the plant's immune system, are transported through the cell and how transport processes control immune responses. PRRs are receptor kinases and receptor-like proteins that must be presented at the plasma membrane to recognize invading pathogens. We found that PRRs from different protein families (FLS2, EFR, PEPR1, Cf-4) are internalized in a ligand-induced and BAK1/SERK3 co-receptor dependent manner and traffic via a common endosomal pathway destined for vacuolar degradation. Clathrin-mediated internalization and sorting by the ESCRT machinery are components of this endosomal pathway and are required for plant immunity. Furthermore, our studies have revealed that inhibition of FLS2 endocytosis leads to de-sensitization to flg22 stimulus. This indicates a link between transport processes involved in PRR trafficking, turnover and defense.

This work is supported by the Gatsby Charitable Foundation and a grant by the European Research Council (ERC).

Towards understanding extra-haustorial membrane-oriented protein targeting and host defense at this host–pathogen interface

S3.4

SHUNYUAN XIAO[1], XIANFENG MA[1], ROBERT BERKEY[1], SHIV KALE[2], YI ZHANG[1], QIONG ZHANG[1], HARLAN KING[1], FLORIAN BITTNER[3], NADINE SCHMIDT[3], WENMING WANG[4]

16:40–17.20

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Despite a great deal of recent advances toward understanding molecular mechanisms of plant immunity, how host defense is executed at the subcellular level (in the host–pathogen interface in particular) remains unclear. Using the Arabidopsis–powdery mildew interaction as the model pathosystem, we demonstrate that the resistance protein RPW8.2 is specifically targeted to the extra-haustorial membrane (EHM) – the host–pathogen interface that encases the fungal feeding structure, i.e. the haustorium. At the EHM, RPW8.2 appears to activate two haustorium-targeted defenses: accumulation of H_2O_2 in and formation of a callosic encasement of the haustorial complex. Our recent work indicates that Arabidopsis xanthine dehydrogenase 1 (XDH1), along with NADPH oxidases RbohD and RbohF, contributes to interface-enriched H_2O_2 for haustorium killing. Interestingly, in leaf mesophyll cells, XDH1 converts xanthine to antioxidant uric acid in local and systemic tissues for removal of H_2O_2 from stress-impacted chloroplasts, thereby protecting plants from stress-induced oxidative damage. Thus, XDH1 appears to play dual and opposing roles in modulation of ROS metabolism during defense responses in Arabidopsis. Our recent studies also show that two basic residue-enriched motifs (R/K-R/K-x-R/K) in RPW8.2 are essential for EHM-targeting and that RPW8.2 preferentially binds phosphatidylinositol 3-phosphate (PI3P), likely via the two EHM-targeting motifs. Since mutations in these two motifs also largely abrogate RPW8.2's binding with PI3P, and PI3P-biosensor proteins appear to localize to the EHM, we suggest that RPW8.2-PI3P binding may establish the polarity of RPW8.2 vesicle trafficking. Finally, by using RPW8.2 and its family proteins as tools, we are interrogating the origin and biogenesis of the EHM induced by powdery mildew in epidermal cells of Arabidopsis.

Session 4: Delivery and function of microbial molecules
Chair: Ton Bisseling

Identifying rust effectors and their roles in disease and immunity

S4.1

PETER DODDS[1], ARWEN ZHANG[1], CLAIRE ANDERSON[2], NADYA FARRAH[2], LAURA ROLSTON[2], STELLA CESARI[1], ROHIT MAGO[1], MAUD BERNOUX[1], SIMON WILLIAMS[3], WENJIE WU[2], ADNANE NEMRI[1], NARAYANA UPADHYAYA[1], BO XU[1], JIAPENG CHEN[4], ROBERT PARK[4], JEFF ELLIS[1], BOSTJAN KOBE[3], ADRIENNE HARDHAM[2], DAVID JONES[2]

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09:00–09.40

Rust fungi such as *Melampsora lini* (flax rust) and *Puccinia graminis fsp tritici* (wheat stem rust) form specialised haustoria structures during infection that serve as nutrient uptake sites as well as delivering effector proteins to the host cell. Four avirulence (Avr) loci are known in flax rust and encode effectors that are recognised by host nucleotide-binding and leucine-rich repeat (NB-LRR) resistance proteins. We have been investigating the molecular basis and cellular location of the recognition events between R and Avr proteins, as well as characterising Avr protein structure and function. Large scale SNP mapping in an *M. lini* F2 family has allowed anchoring of the genome sequence onto a genetic map and identified several additional Avr genes, and expression profiling reveals a common pattern for a subset of effector candidates including all of the known *Avr* genes. We also seek to extend observations from the flax rust system to the important wheat stem rust disease, which is a major threat to global food security. We recently identified the stem rust resistance genes *Sr33* and *Sr50*, which encode CC-NB-LRR proteins closely related to barley MLA powdery mildew resistance proteins. However, structural and functional analyses reveal several important differences from MLA. While both proteins trigger cell death through their CC domains, the truncated domain corresponding to the structurally characterised MLA CC dimer, is not active and does not form dimers. Analysis of stable wheat transgenic plants expressing Sr33-YFP fusions with nuclear localization or export signals showed that Sr33 triggers both cell death and defence responses from a cytosolic but not nuclear location. Mutational analysis of wheat stem rust has identified several candidates for the *AvrSr50* effector recognised by *Sr50*.

The secreted effector repertoire of smut fungi and finding out where they function

S4.2

LIBERA LO PRESTI, LIANG LIANG, DANIEL LANVER, NICOLE LUDWIG, STEFANIE REISSMANN, REGINE KAHMANN

09:40–10.20

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

Smut fungi comprise a large group of biotrophic pathogens that infect cereal crops and wild grasses. The best studied member of this group, *Ustilago maydis*, infects maize and induces characteristic tumor formation and anthocyanin induction. During host colonization, *U. maydis* establishes an extended interaction zone with the plant in which fungal hyphae are encased by the host plasma membrane. Interaction with the plant is largely determined by protein effectors that are conventionally secreted. A successful colonization requires active effector-mediated suppression of plant defense responses and host tissue reprogramming. Secreted effector proteins can either display their activity in the apoplast or translocate to host cells. While bacterial pathogens use Type III secretion systems for injecting effectors into plant cells, the molecular mechanism of effector delivery by eukaryotic plant pathogens remains elusive. In addition, we do not yet know what determines effector uptake by plant cells or retention in the apoplast. One of the problems encountered in the *U. maydis*/maize system is that translocated effectors carrying fluorescent protein tags cannot be detected in the host cell by their fluorescence. Here we report the establishment of an uptake assay that is based on the ability of a bacterial biotin ligase, BirA, to biotinylate proteins *in vivo* that carry a small peptide tag (AviTag). The assay relies on transgenic maize plants expressing BirA in the cytosol and infecting these plants with *U. maydis* expressing effector-AviTag fusion proteins. The only effectors to be biotinylated by BirA should be the ones that translocate into the host cytoplasm. Biotinylation should thus become a hallmark of uptake. I will discuss our results for a number of apoplastic and cytoplasmic *U. maydis* effectors.

JAZ proteins in poplar roots: a checkpoint for establishment of mutualistic ectomycorrhizal interactions?

S4.3

CLAIRE VENEAULT-FOURREY[1,2], YOHANN DAGUERRE[1], CLEMENT PELLEGRIN[1,2], FENG ZHANG[1], ROMAIN SCHELLENBERGER[1,2], ANNEGRET KOHLER[1], SEBASTIAN WITTULSKY[1], JONATHAN PLETT[3], FRANCIS MARTIN[1]

11:10–11.50

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In forest ecosystems, hyphae of ectomycorrhizal (ECM) fungi constitute a significant proportion of soil microbial biomass. Soil-borne hyphae interact with tree roots to form ectomycorrhizal symbiosis, providing N, P nutrients to the tree in return for photosynthetically-derived carbon from their hosts. While colonization of roots by ECM fungi is invasive leading to dramatic morphogenetic changes of roots and cell wall remodelling, weak plant defence responses are triggered. We showed that, similarly with plant-pathogenic microbes, the ectomycorrhizal fungus *Laccaria bicolor* use secreted effector proteins to establish mutualistic interaction. In particular, the small mycorrhiza-induced secreted protein MiSSP7 is counteracting one part of the jasmonic acid (JA) signalling through its interaction with *Populus trichocarpa JAZ6* protein, a putative co-receptor of jasmonic acid [1]. I will discuss our most recent findings concerning the role of JAZ proteins in the symbiosis development and poplar cellular biology. I will also summarize our ongoing studies on apoplastic effectors of *L. bicolor*, highlighting how the study of effectors from mutualistic fungi will provide a better understanding of plant physiology.

1. Plett JM, Daguerre J et al., (2014), *PNAS*; 111 (22), 8299–8304

Effector delivery by the blast fungus during biotrophic invasion of rice

S4.4

11:50–12.30

BARBARA VALENT[1], ELY OLIVEIRA-GARCIA[1], HUAKUN ZHENG[1,5], MIHWA YI[1,6], PIERRE MIGEON[1], MELINDA DALBY[1], QINGYU WU[2], KIM PARK[2], SUNHUNG PARK[2], WANG XU[3], JUNG-YOUN LEE[3], MARK FARMAN[4], ZONGHUA WANG[5], and JIE ZHOU[5]

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The hemibiotrophic rice blast fungus *Magnaporthe oryzae* differentiates specialized invasive hyphae (IH) that grow extensively inside living rice cells and then spread in surrounding neighbor cells to eventually form typical eyespot lesions. Invasive hyphae grow while enclosed in host-derived extrainvasive-hyphal membrane (EIHM). Immediately after entry into a host cell, the fungus undergoes a characteristic morphological switch, and the IH cells that undergo this switch are associated with a specialized interfacial structure, the biotrophic interfacial complex (BIC). Cytoplasmic effectors accumulate in BICs via a Golgi-independent secretion system involving the exocyst complex, while apoplastic effectors are secreted via the classical Golgi-dependent secretion system in the nonBIC-associated IH cells that subsequently grow to fill the host cells. Our current working hypothesis is that host translocation of cytoplasmic effectors is a two-step process that first involves secretion and accumulation in BICs and then translocation across the EIHM into the host cytoplasm. We will present new live cell imaging showing cytoplasmic effectors that appear packaged into vesicles in and around BICs, and suggesting internalization into rice cells by endocytosis. At least 30 BIC-localized effectors are translocated into the cytoplasm of invaded rice cells and move ahead into neighboring rice cells, presumably moving through plasmodesmata, and we have evidence suggesting that IH seek out pit fields before crossing into neighboring cells. We explore potential fungus–plasmodesmata interactions using 7 distinct effectors that accumulate around hyphae as they cross the plant cell wall, and other effectors that undergo cell-to-cell movement.

Session 5: Cell surface response
Chair: Sophien Kamoun

Membrane trafficking in plant cells attacked by powdery mildew fungi **S5.1**

HANS THORDAL-CHRISTENSEN[1, 2], MADS E. NIELSEN[1] & MARK KWAAITAAL[2] **13:50–14.30**

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We are interested in plant membrane trafficking processes and how they are involved in plant interactions with microbes. For this purpose we use the powdery mildew fungi and the attacked leaf epidermal cells, which are amenable for confocal microscopy. These fungi are obligate biotrophs, introducing haustoria in the host cell as a means of acquiring nutrients.

We have previously shown that the syntaxin, PEN1, is required for plant defence against penetration and haustoria establishment. Later, we found that treatment with Brefeldin A (BFA) hampers penetration resistance. BFA suppresses a subset of the plant's ARF GTPase guanine nucleotide exchange factors (GEFs), which regulate membrane budding. A well-studied BFA-sensitive ARF GEF is GNOM. By introducing a mutant version of GNOM, which is insensitive to BFA, we could show that GNOM is the ARF GEF that is essential for penetration resistance. A defence component, which is often observed but poorly studied, is the haustorial encasement. Here a cell wall-like structure forms around the haustorium. We have uncovered a Rab GTPase involved in encasement formation and for the first time been able to document that this structure suppresses pathogen proliferation. Pathogen haustoria are surrounded by plant-generated membranes. The nature of these extrahaustorial membranes (EHM) remains enigmatic. We have addressed this question and found that the powdery mildew-associated EHM in barley cells shares features with the endoplasmic reticulum membrane (ER), although the EHM is not an extension of the ER.

Forward genetic screening reveals new insights into cellular processes governing local recruitment of defenses to the plant–microbe interface

S5.2

BILL UNDERWOOD[1,2], ANDREW RYAN[1,3], SHAUNA SOMERVILLE[1]

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14:30–15.10

Upon attack by microbial pathogens, plant cells initiate a highly-localized defense response that includes local reinforcement of the cell wall and likely also involves the synthesis and targeted export of antimicrobial metabolites. This focal response is initiated upon detection of conserved pathogen elicitors referred to as pathogen-associated molecular patterns (PAMPs). Perception of these elicitors triggers the recruitment of proteins that contribute to local defenses at the cell surface, including the Arabidopsis PEN1 SNARE protein and the PEN3 ABC transporter, both of which are required for full resistance to penetration of the cell wall and establishment of haustoria by inappropriate powdery mildew fungi. PAMP perception is sufficient to initiate recruitment of PEN1 and PEN3, implying that cell surface pattern recognition receptors (PRRs) responsible for PAMP detection can convey spatial information required for proper positioning of physical and chemical cell wall reinforcements. However, the mechanisms through which PRRs impart the spatial information of pathogen detection at the cell surface and the cellular processes governing recruitment of defense-related proteins to the host–pathogen interface are poorly understood. To address these questions, we conducted a forward genetic screen to isolate Arabidopsis mutants that mis-target the PEN3 transporter upon challenge with an inappropriate powdery mildew. I will discuss our progress toward mapping and characterizing these mutants including insights gained from characterization of a mutant disrupted in a trans-Golgi network-localized lipid flippase.

Virulence strategies of the fungal biotrophs: lessons from the *Ustilago maydis*–maize model system

S5.3

GUNTHER DÖHLEMANN

16:20–17.00

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CEPLAS/Institute of Botany, University of Cologne, Germany

The fungus *Ustilago maydis* causes the formation plant tumors on all aerial organs of its host plant maize. *U. maydis* establishes a biotrophic interaction directly upon host penetration and growth intra- as well as intracellularly without eliciting visible defense reactions. This type of infection requires an efficient suppression of plant defense responses. Recent findings suggest that conserved components of the apoplastic plant defense machinery are direct targets of secreted *U. maydis* virulence factors, so-called effector proteins. Important plant targets of effectors are papain-like cysteine proteases, which are crucial for the activation of salicylic-acid (SA) dependent defense reactions in maize. To understand how apoplastic proteases trigger downstream immune responses, we searched for signals that could be released by protease activity. A mass spectrometry approach detected maize peptides, which are accumulating upon protease activation. One of these peptides was found to activate maize defense gene expression, indicating the identification of a novel apoplastic immune signal in maize.
A striking feature of *U. maydis* is its ability to cause tumors in all aerial parts of its host plant. In light of the huge differences between these maize tissues that are transformed to tumors, we hypothesized that *U. maydis* deploys organ specific effectors to manipulate physiology and development of infected tissues. Using a gene-deletion strategy, we could novel *U. maydis* effectors, which contribute to virulence in an organ-specific manner. A combination of microscopy and biochemical analyses are used to functionally characterize these new effectors and to understand molecular basis of tissue-specificity. This approach demonstrates how pathogen effectors serve as molecular probes to elucidate molecular mechanisms of host-microbe interaction.

The formation of an infection-related membrane domain is controlled by the sequential recruitment of scaffold and receptor proteins

S5.4

T.F. STRATIL, C. POPP, S.S.A. KONRAD, M. MARÍN, J. FOLGMANN, T. OTT

17:00–17.40

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Intracellular colonization of plant cells by symbiotic bacteria is a critical step for the host that requires stringent surveillance circuits at the plasma membrane to keep exclusive control over the infection process. Accumulating evidence suggests that such perception and signal transduction complexes are pre-formed in membrane compartments such as mesoscale membrane domains (MMDs). However, neither the existence of pathway-specific MMDs nor their controlled assembly has been demonstrated. Here, we unravelled the sequential organization of membrane-resident signalling proteins that are indispensable for the intracellular infection of *Medicago truncatula* roots by symbiotic bacteria. We identified actin, the flotillin FLOT4, the remorin SYMREM1 and the entry receptor LYK3 as essential molecular building blocks that are required and sufficient for the assembly of an infection-related MMD in vivo. Reciprocally, the combinatorial expression of these proteins in a heterologous cell system was sufficient to artificially reconstitute this specialized membrane domain *in vivo*.

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Poster Abstracts

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P1 HopZ1a targets a remorin implicated in membrane-associated defence signalling

P. ALBERS[1], S. ÜSTÜN[1], F. BÖRNKE[1,2]

[1]Leibniz-Institute for Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany; [2]Institute for Biochemistry and Biology, University of Potsdam, Germany

HopZ1a, a member of the YopJ superfamily from *Pseudomonas syringae*, was shown to display acetyltransferase activity towards tubulin leading to the inhibition of secretion during defense responses. To identify new HopZ1a targets we initiated a yeast-two-hybrid (Y2H) screen and identified a remorin as interactor of HopZ1a which we named HopZ1a-interacting-protein 1 (HIR1). To characterize a role of HIR1 in plant immunity, we performed additional Y2H screens and identified PBS1, a protein kinase involved in plant defence, and SINA4, an E3 ubiquitin ligase as part of a putative HIR1 interactome. Using split-YFP, we confirmed the interaction of HopZ1a and HIR1, as well as HIR1 and PBS1, with both complexes associating at the plasma membrane. Cell biological approaches revealed that upon flg22 treatment HIR1 shifts into punctuated structures at the plasma membrane resembling lipid rafts. Furthermore, preliminary results indicate a role of HIR1 as a positive regulator of PTI, as PTI marker gene expression is increased in plants overexpressing HIR1 and ROS production is affected in plants silenced for HIR1. In summary, our findings support the hypothesis that HIR1 might act in a complex together with immune kinase PBS1 during PTI and hence is targeted by HopZ1a to manipulate membrane-associated defence responses.

P2 A bacterial effector targets host plasmodesmata to promote pathogen virulence in plants

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

The *Arabidopsis–Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 pathosystem has been extensively studied to elucidate basic principles underlying plant–microbe interaction; however, little is known whether bacteria modulate adjoining non-infected plant cells for successful infection. Recent reports demonstrated that plants induce plasmodesmatal closure as part of a defense response against bacterial infection. *Pst* DC3000 injects ~36 virulence effector proteins into plant cells to subvert plant immunity. We discovered that *Pst* DC3000 effector HopO1-1 is targeted to the plasmodesmata in *Arabidopsis*. Expression of HopO1-1 in *Arabidopsis* leads to an increase in PD-dependent molecular flux between plant cells. Moreover, HopO1-1 is physically associated with PD-located protein 5 (PDLP5), which is previously shown to be involved in plant immunity, and PDLP7. Consistent with its predicted function, we demonstrated that HopO1-1 is an active ADP ribosyltransferase enzyme. Our results suggest that bacterial pathogens deliver effectors such as HopO1-1 to modulate host plasmodesmatal function, presumably by ADP-ribosylating PDLPs, to facilitate bacterial spread and multiplication.

P3 Role of cell-to-cell communication during the establishment of the nitrogen-fixing symbiosis

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The establishment of the nitrogen-fixing endosymbiotic interaction between *Medicago truncatula* and *Sinorhizobium melliloti* involves major and specific host cell reprogramming as the infecting microbe progresses across outer root cell layers to reach the inner cortical nodule primordium. Accumulating evidence suggests that direct cell-to-cell communication plays a central role in this highly specialized transcellular infection process which involves the formation of successive apoplastic 'infection thread' compartments. Unique cell wall channels in plants, so-called plasmodesmata (PDs), are essential for direct cell-to-cell communication and we are therefore studying PD distribution and symplastic fluxes during early symbiotic infection stages by using *in vivo* markers and specific staining protocols. Furthermore, *In silico* analysis of *Medicago* transcriptomic data sets have identified several candidate genes upregulated in the *Medicago* nodule infection zone coding to putative PD-associated proteins. This includes 5 genes encoding β-1,3-glucanases that are closely related to known *Arabidopsis* β-1,3-glucanases which regulate PD flux during root development as well as 1 PD-localized-protein and 2 PD-callose-binding-proteins. Their expression and subcellular localization are currently being investigated. Results and their putative role during infection will be presented and discussed.

P4 *In silico* discovery of *Verticillium nonalfalfae* virulence factors and their functional verification

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Verticillium wilt of hops has emerged as a serious threat to hop production in Europe due to outbreaks of highly aggressive lethal strains of *Verticillium nonalfalfae*. This soil-borne fungus invades plant roots and enters xylem vessels, passively spreading with xylem sap throughout the entire plant. Subsequently, wilting symptoms develop and vascular browning, foliar chlorosis, necrosis and plant death are observed.
Using modern high-throughput technologies to study molecular mechanisms of pathogenesis of *V. nonalfalfae* in hop, we have gathered a huge amount of experimental data. Meaningful interpretation of such data is challenging and usually requires some computing knowledge. To this end, we employed the web platform GenCloud, which not only stores data in a cloud and provides easy access and distribution of data but also enables interactive user-friendly processing of data in customized bioinformatic pipelines. We thus characterized and functionally annotated gene sequences of *V. nonalfalfae.* We then determined *in silico* the fungal secretome that probably encompasses the virulent factors (e.g., toxins, enzymes and effectors) that modulate plant immune responses and contribute to successful colonization. Finally, we verified the biological role of selected fungal candidate effectors with expression analysis during invasion of resistant and susceptible hop cultivars and assessed the virulence of effector knock-out mutants in pathogenicity tests.

P5 Molecular determinants of the VgrG proteins governing type VI effector transport specificity

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In the type VI secretion system (T6SS), a macromolecular machine forming a bacteriophage tail-like structure, the valine glycine repeat G (VgrG) protein has been suggested to function as a puncturing device and transporter of specific effectors. However, the molecular determinants and underlying mechanisms of the effector transport specificity of the cognate VgrG protein remains to be elucidated. Here, we report that two *vgrG* paralogs in *Agrobacterium tumefaciens* C58 specifically control the secretion and *in planta* interbacterial competition activity of the type VI DNase toxins Tde1 and Tde2. Deletion and domain swapping analysis identified that the C-terminal extension unique to VgrG1 specifically confers secretion and Tde1-dependent interbacterial competition activity *in planta*, and the C-terminal variable region of VgrG2 governs this specificity for Tde2. Further functional studies of VgrG1 and VgrG2 variants with stepwise deletion of the C-terminus revealed that the C-terminal 31 aa (C31) of VgrG1 and 8 aa (C8) of VgrG2 are molecular determinants specifically required for delivery of each cognate Tde toxin. Further sequence and bioinformatics analyses revealed a striking conservation of cognate *vgrG* genetically linked with distinct chaperone/adaptor and effector genes in Proteobacteria. These results suggest that the divergent C-terminal end of VgrG protein has evolved a conserved function governing specificity of effector delivery.

P6 An effector from Irish potato famine pathogen mediates selective autophagic cargo sorting

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Autophagy contributes to immunity both in animals and plants. A form of selective autophagy, organized by autophagy cargo receptors, protects mammalian cells against infection. However, we know little about how selective autophagy is regulated at the molecular level, particularly how it contributes to immunity in plants. Previously, we discovered that PexRD54, an effector secreted by *Phytophthora infestans* subverts autophagy related host defenses. PexRD54 binds host autophagy regulator ATG8CL through its ATG8 interacting motif and stimulates autophagosome formation. Yet, how PexRD54 manipulates host autophagy is poorly understood.
Here we investigated mechanisms of autophagosome formation and potential cargo sorting mediated by PexRD54. We hypothesized that PexRD54 stimulates autophagosome formation; possibly through recruiting other host components to ATG8CL coated pre-autophagosomal membrane compartments. We show that PexRD54 interacts with Rab8, a member of small GTPases that mediate vesicle transport. PexRD54-Rab8 association was independent of PexRD54-ATG8CL binding, since a PexRD54 mutant that cannot bind ATG8CL still interacted with Rab8. Furthermore, PexRD54 colocalized with Rab8 and stimulated recruitment of Rab8A to ATG8CL coated autophagosomes. Conversely, Rab8A did not bind host autophagy cargo receptor Joka2 and is excluded from Joka2 labeled autophagosomes. The role of Rab8 in autophagy and immunity will be discussed.

P7 Looking for symbiosis-related effector proteins in the ericoid endomycorrhizal fungus *Oidiodendron maius*

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Ericoid mycorrhizal (ERM) fungi form endomycorrhiza with plants in the family Ericaceae. Among ERM fungi, *Oidiodendron maius* (Ascomycota, class Leotiomycetes) has been developed as a model system to investigate the molecular bases of the ERM symbiosis. The genome and the transcriptome of *O. maius* have been searched for genes coding for small secreted proteins (SSPs) that may act as effectors in ERM. Effectors corresponding to SSPs have been reported for both AM and ECM fungi. 445 genes encoding SSPs were found in the *O. maius* genome. Transcriptomic analyses revealed that, among them, 89 (20%) are over-expressed in symbiosis, the most up-regulated (SSPb, ID: 182936) being expressed over 20,000 times. RT-qPCR confirmed over-expression of SSPb in mycorrhizal roots, when compared to the free living mycelium. The SSPb transcript codes for a protein that share some features with hydrophobins. Hydrophobins are small secreted morphogenetic fungal proteins produced in response to changes in developmental and environmental conditions. These proteins are important in the interaction between certain fungi, such as ECM fungi, and their hosts.
Bioinformatic analyses revealed similarities of SSPb with other fungal hydrophobins, but our preliminary data do not indicate a localization of SSPb in the cell wall.

P8 The CCaMK/CYCLOPS complex regulates lateral root organ formation in *Lotus japonicus*

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Plant productivity depends on the adaptation of the root system architecture to uneven nutrient distribution and inhomogeneous soil structure. Interactions with arbuscular mycorrhiza fungi or lipo-chitooligosaccharide signals from rhizobia modulate root system architecture (Maillet *et al*., 2011; Paszkowski and Boller, 2002; Oláh *et al*., 2005). Although the molecular mechanisms that regulate nodule and lateral root initiation have been extensively studied in legumes and in *Arabidopsis*, respectively, the connection between the symbiotic signalling pathway and that of lateral root initiation remains to be elucidated. We found that dominant active version of CCaMK or CYCLOPS can stimulate lateral root formation in *Lotus japonicus*. This system provides a genetic approach towards the identification of the mechanisms by which signals from microsymbionts modulate the root architecture of the host plant.

Maillet *et al*. (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature*, **469**: 58–63.
Oláh *et al*. (2005) Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. *Plant Journal*, **44**: 195–207.
Paszkowski and Boller (2002) The growth defect of *lrt1*, a maize mutant lacking lateral roots, can be complemented by symbiotic fungi or high phosphate nutrition. *Planta*, **214**: 584–590.

P9 Changes in epiphytic orchid mycorrhizal fungi from two altitudinal levels in tropical rainforest in southern Ecuador

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Orchids depend on nutrients, particularly carbon, supplied by mycorrhizal fungi for seed germination and early seedling development. Mycorrhizal fungi of *Tulasnellales*, *Sebacinales*, *Ceratobasidiales* and *Atractiellales* have been found in green epiphytic and terrestrial orchids of tropical forests in South America. However, the vast diversity of these organisms and their interactions with orchids has not been fully studied yet in the tropics. We hypothesized that orchid mycorrhizal fungi are widely distributed in tropical ecosystems and are not a limiting factor for orchid establishment. We used Illumina sequencing to detect the mycorrhizal OTUs associated to three epiphytic orchids shared between two tropical forests in Southern Ecuador. After sequences analysis, 422 fungal OTUs were detected including 54 OTUs of orchid mycobionts. *Ceratobasidium* was the largest genus with 23 OTUs besides *Sebacina* with 22 and *Tulasnella* had nine. Seven OTUs were shared among all orchid species regardless the site, while the remaining 45 OTUs of orchid mycobiont had limited occurrence among orchids. That is, they were present in one orchid species but not in all. New Generation Sequencing gives a wide overview of orchid associated fungal communities that most of the time passes unnoticed.

P10 Identification of SA-signaling inhibitors and their target protein in *Arabidopsis*

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To identify chemical compounds which alter *Arabidopsis* immune responses, we developed a high-throughput screening method for hypersensitive response (HR) cell death induced by *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 *avrRpm1*. Among a large number of chemical compounds involved in HR cell death, we identified a compound designated as P7, which inhibits *Arabidopsis* immune responses. *Arabidopsis* treated with P7 suppressed expression of *PR1*, the salicylic acid (SA) marker gene, and nuclear accumulation of NPR1, the key regulator of SA-mediated gene expression, in response to SA treatment. These results suggest that P7 is an inhibitor for the SA signaling pathway. Furthermore, treatment with P7 also suppressed the induction of PR1 in response to *Pst* DC3000 and significantly increased susceptibility to *Pst* DC3000. In addition, we have identified a CUPIN domain containing protein, designated as CUPIN1, as a target of P7 in *Arabidopsis*. A point mutation in CUPIN1 reveals its role in SA responses. The possible role of CUPIN1 in plant immune signaling will be discussed.

P11 Functional characterisation of the *Cercospora zeina crp1* gene as a putative pathogenesis regulation factor

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Grey leaf spot (GLS) is a devastating maize foliar disease caused by *Cercospora zeina* in South Africa. The fungal circadian clock regulates various aspects of pathogenesis including the fungus' ability to perceive and orientate growth towards the plant's stomata. The limiting component of the circadian clock, as identified in *Neurospora crassa*, is the putative blue-light receptor, White Collar-1 (WC-1). The *Cercospora* Regulator of Pathogenesis (*crp1*) gene from *Cercospora zeae-maydis* encodes putative blue-light receptor homologous to the WC-1 protein. Functional characterisation of the CRP1 protein in *C. zeina* was performed through gene disruption with split marker constructs, which replaced the *crp1* gene with a hygromycin resistance gene. The generated transformants were cultured and screened for the presence and correct genomic location of the hygromycin resistance gene. The inability of the knockout mutants to sense blue light contributed to the fungus forming a higher concentration of conidia in the presence of light, compared to the wild type *C. zeina,* which could only effectively produce conidia in the dark. Additionally, the disrupted gene altered the manner in which melanin was deposited in the *crp1* knock-outs. Pathogenesis of the *Δcrp1 C. zeina* cultures was tested through a maize infection trail and subsequent confocal microscopy revealed a reduction in infection structure formation compared to the wild type. In conclusion, these experiments suggest that crp1 plays a role in the pathogenesis of *C. zeina* on maize.

P12 Subversion of autophagy by the Irish potato famine pathogen *Phytophthora infestans*

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Autophagy is a multifaceted membrane trafficking pathway involved in adaptation to cellular stress conditions. Activation of autophagy leads to formation of special vesicular structures called autophagasomes, which carry autophagic cargo to lysosomes or vacuoles for degradation. A form of autophagy, known as selective autophagy, can specifically degrade toxic substances such as pathogens. Selective autophagy functions through autophagy cargo receptors that define autophagic cargoes. The role of autophagy in plant–microbe interactions is unclear. Here, we discovered that a secreted RXLR-WY type effector of *Phytophthora infestans*, named PexRD54, binds to the autophagy marker protein ATG8 via an **A**TG8 **I**nteracting **M**otif (AIM). PexRD54 did not have a negative effect on autophagic flux and stimulated autophagosome formation. To investigate the biological function of PexRD54, we studied the autophagy cargo receptor Joka2, which also interacts with ATG8. Overexpression of Joka2 *in planta* limited *P. infestans* infection, suggesting a role for Joka2/ATG8 selective autophagy in response to oomycete infection. Remarkably PexRD54, but not the AIM mutant of PexRD54, was able to out-compete Joka2 for binding to ATG8 and restore full pathogen virulence. Our findings point to a model in which an RXLR-WY effector from *P. infestans* antagonizes a selective autophagy cargo receptor to enhance pathogen virulence.

P13 A cell biology view on the susceptible interaction between plants and obligate sedentary parasitic root-knot nematodes

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Sedentary endoparasitic root-knot nematodes (*Meloidogyne* spp.) are competent to modify plant root cells by inducing specialized feeding structures. They provoke changes in selected root vascular cells to form complex feeding cells that supply nutrients for the nematodes to enlarge, become sedentary and to finally develop into fertile adults females. Feeding cells induced by root-knot nematodes are characterized by a dense cytoplasm filled with proliferating organelles. Complex changes that occur during feeding cell morphogenesis are accompanied by a drastic rise in ploidy levels, metabolic activity and cell size. We have shown that activation of the cell cycle as well as reorganization of the cytoskeleton play key roles in feeding site development. How precisely nematodes manipulate these processes in their favour remains to be understood. A systematic comparison of the temporal and spatial expression pattern of core cell cycle genes between galls and uninfected control roots of *Arabidopsis thaliana* resulted in the identification of a collection of genes up- or downregulated in nematode feeding sites. Functional analyses of selected genes are on course and resulted in the identification of a subset of genes strongly implicated in gall development. Concomitant with cell cycle activation, a reorganization of the actin and microtubule network occurs in targeted vascular parenchymal root cells upon nematode infection. Genes involved in these reorganizations have been identified including major genes like gamma tubulins and actin-depolymerizing factors (ADF). To better understand giant cell development, *in vivo* subcellular observations of the cytoskeleton and localization studies of cell cycle proteins in feeding cells have been carried out.

P14 Membrane traffic components involved in MAMP-triggered callose accumulation in Arabidopsis

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Plants are known to generate a structural barrier known as “papillae”, which consists of a beta-1,3-linked glucan, callose and other components, at the tip of invading fungal hyphae as a part of immune response. It has been indicated that the host membrane traffic system plays important roles in the callose deposition at the specific invasion site but the details of this process is still unclear. Knowing that similar callose deposition could also be induced by various MAMP treatments, we tried to identify the components involved in the membrane traffic required for MAMP-induced callose deposition. For this purpose, we developed a simple computer program by which we could quantify the callose spots on the leaves treated with MAMPs, avoiding time-consuming manual counting as well as the errors originated from personal judgment. By using this approach, we evaluated MAMP-induced callose deposition in various mutants for membrane traffic and found those genes involved in up or down regulation of the callose deposition.

P15 Identifying mechanisms of nutrient transport from plants to biotrophic pathogens

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Successful plant pathogens must execute two tasks. The first task, suppression of plant immunity, is studied intensively and is increasingly well understood. The second task, equally important but less understood, is to acquire nutrients from the plant host. Evidence suggests that plant pathogens reprogram host nutrient biosynthesis and transport pathways. However, little is known about the mechanisms through which oomycetes extract nutrients from susceptible host plants. Our project aims to define how oomycetes accomplish this, with emphasis on identifying host susceptibility genes. Specifically, we are developing two methodologies: the first focuses on high spatial/ temporal resolution transcriptomics to identify genes active in a sub-population of cells, using translating ribosome immunopurification technology. The second allows for quantitative measurement of nutrient transfer from plant to pathogen using sensitive instruments housed at Virginia Tech. Both technologies will be used to identify and study the genes co-opted by pathogens during feeding.
The long-term goal of this project is engineering of these genes in crops so that they are not available to pathogens, effectively cutting pathogens' supply lines and preventing disease. In principle, this approach will provide resistance against a wide range of pathogens and would be very difficult for pathogens to overcome by co-evolution.

P16 The role of Synaptotagmin1 in the formation of symbiotic interface

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The entrance of microsymbiont into a host cell causes the formation of specific for symbiosis protrusions of plasma membrane like infection threads, unwalled droplets and arbuscules. We hypothesized that the expansion of these structures causes membrane stretch which may serve as a vector for exocytosis targeted to the interface membrane. To test this hypothesis, we studied *MtSyt1, MtSyt2* and *MtSyt3, Medicago truncatula* homologs of synaptotagmin 1. This membrane trafficking protein is functional as Ca^{2+} sensor in SNARE-dependent plasma membrane vesicle fusion and membrane repair. Promoter-GUS analysis has shown that *MtSyt1, MtSyt2* and *MtSyt3* are expressed in nodule primordia and apical parts of root nodules. The localization of GFP-tagged MtSyt1, MtSyt2 and MtSyt3 at the site of bacterial release, around infection threads and arbuscules supports the hypothesis that synaptotagmins are involved in targeted exocytosis toward symbiotic interface. The double silencing of *MtSyt2* and *MtSyt3* in root nodules shows that synaptotagmins are operational in the growth of infected cells and intracellular rhizobia accommodation.

P17 Infection chamber remodeling allows rhizobial entry into *Medicago truncatula* root hairs

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In *Medicago truncatula*, controlled root entry of symbiotic nitrogen-fixing rhizobia takes place in root hairs (RHs) via tubular tip-growing structures known as infection threads (ITs). ITs develop following bacterial entrapment in curled RHs within a space called the infection chamber. A live-tissue imaging approach using fluorescent protein fusions to detect exocytosis sites and host cell wall remodeling revealed that tubular IT development in curled RHs only initiates after a phase of active radial expansion and remodeling of the infection chamber wall/matrix interface by the RH cell, concomitant with rhizobial multiplication. This key phase of infection chamber remodeling is dependent on the Nodule Inception symbiotic transcription factor. Monitoring host symbiotic Ca^{2+} responses (spiking) using a nuclear-localised cameleon Ca^{2+} reporter also showed that infection chamber remodeling correlates with a severe attenuation in RH Ca^{2+} spiking, which is later re-activated during the initiation of the tubular IT growth. We therefore propose a 2-step model for rhizobial entry in legume RHs in which the infection chamber first develops into a globular apoplastic compartment displaying structural similarities to the future IT and hosting the bacterial micro-colony. A switch to polar elongation, concomitant with a re-activation of Ca^{2+} spiking, then leads to tubular IT initiation.

P18 The phosphate transporters LjPT4 and MtPT4 mediate early root responses to phosphate status in non mycorrhizal roots

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Arbuscular mycorrhizal (AM) symbiosis improves host plant phosphorous (P) status and elicits the expression of AM-inducible phosphate transporters (PTs) in arbuscule-containing cells, where they control arbuscule morphogenesis and P release. We confirmed such functions for *LjPT4* in mycorrhizal *Lotus japonicus*. Promoter-GUS experiments showed *LjPT4* transcription not only in arbusculated cells, but also in root tips, in the absence of the fungus: here *LjPT4* transcription profile depended on the phosphate level. In addition, quantitative RT-PCR confirmed the expression of Lotus and *Medicago truncatula PT4* in the tips of non-mycorrhizal roots. Starting from these observations, we hypothesized that AM-inducible PTs may have a regulatory role in plant development, irrespective of the fungal presence. Firstly, we focused on root development responses to different phosphate treatments in both plants demonstrating that phosphate starvation induced a higher number of lateral roots. By contrast, Lotus PT4i plants and Medicago *mtpt4* mutants did not show any differential response to phosphate levels, suggesting that *PT4* genes affect early root branching. Phosphate starvation-induced genes and a key auxin receptor, MtTIR1, showed an impaired expression in *mtpt4* plants. We suggest that *PT4* proteins may integrate signals from the plant phosphate status to regulate root branching, independently of AM fungi.

P19 Effector gene expression in *Plasmopara viticola* strains with different virulence against a tolerant host

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The obligate biotrophic oomycete *Plasmopara viticola* is a destructive pathogen of grapevine (*Vitis vinifera*). Resistance breeding has generated grapevine cultivars, such as 'Regent' tolerant to the pathogen. However, strains of the downy mildew have been found which are able to overcome this resistance. A phenotypic evaluation system based on leaf disc infection tests with different host genotypes allowed us to select specific host–pathogen combinations for studying mechanisms involved in successful infection. Fluorescence microscopy studies confirmed at the tissue level the observations made in the bioassay. Expression patterns with putative effector genes in specific combinations indicate which genes are involved in the pathogenesis of the oomycete. Among the studied genes, one bearing a RXLR motif presented a very high expression in a strain highly virulent to 'Regent' in comparison to a low virulent strain. The highest effector expression was found at 6 h post inoculation, emphasizing the importance of an early up-regulation to achieve a successful infection. The gene expression of *in vitro* germinated spores revealed the fact that upregulation of these genes takes place even before a contact has been established between the pathogen and the plant.

P20 Strawberry genotypes that suppress the emergence of spotted-wing fly (*Drosophila suzukii*) from ripe fruits

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Drosophila suzukii is threatening soft fruit production worldwide due to the female's ability to penetrate the outer tissue layer and lay eggs into intact ripening fruits. This is typically associated with microbial infection of the damaged fruits. Drastic economic losses caused by *D. suzukii* outbreaks have been recorded in soft fruit production such as cherries. Ripe fruits must not be treated with insecticides because of their toxicity for humans although they are the favored oviposition location by the flies. Ideally cultivars could be produced that do not support fly development, thus do not contribute to propagation of the pest. In order to achieve this goal, we analyzed the fly emergence from fruits of 107 *Fragaria* accessions (cultivars, hybrids, and wild collections) encompassing 12 species. The degree of *D. suzukii* compatibility was quantified as the number of flies emerging from fruits exposed to egg-laying females. We observed significant differences in *D. suzukii* emergence between these accessions. The degree of fly emergence correlated with ploidy level (decaploid being an exception), but not with fruit size. Eleven emergence suppressing candidate accessions belonging to *F. vesca, F. nilgerrensis, F.* x*bifera* and *F. moschata* were identified, serving as valuable sources to identify underlying resistance genes.

P21 Regulation of arbuscule development

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Root colonization by arbuscular mycorrhiza (AM) culminates in the formation of highly branched fungal arbuscules, which release mineral nutrients to the host. Arbuscule formation is accompanied by drastic subcellular rearrangements of the accommodating root cortex cells. It is poorly understood how these rearrangements are regulated and executed at the molecular level. Using forward genetics coupled with epistasis analysis we have revealed a transcriptional cascade in *Lotus japonicus* that is required for arbuscule development.

P22 Functional analysis of mycorrhiza-related GRAS transcription factor genes in *Medicago truncatula*

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The establishment of a functional arbuscular mycorrhizal (AM) symbiosis between *Medicago truncatula* and *Rhizophagus irregularis* is governed by a specific continuous transcriptional reprogramming. This reprogramming includes the activation of genes encoding transcription factors (TFs), located at the terminus of a signal cascade that ultimately leads to the expression of AM-related genes. Previous genome-wide gene expression analyses revealed a set of GRAS-TFs being specifically upregulated primarily during later stages of AM symbiosis. To sharpen the view on these TF genes, potentially controlling mycorrhization, transgenic roots expressing knockdown constructs are generated and investigated, focusing on their influence on the differential transcription of AM-related and signaling-related genes. Since TFs are often part of dimers, interdependencies between the chosen TFs are conceivable. To investigate possible interplays between the TFs, yeast two-hybrid screenings have been initiated. These screenings might lead to the identification of binding partners that might compensate for the knockdown of single distinct TFs and prevent the establishment of phenotypes in knockdown/knockout mutants. If there are such interplaying TFs, those will be targets of a post transcriptional co-knockdown, in order to enforce a physiological response that allows inferences about the TF's function during AM symbiosis.

P23 Phenotyping of early plant defense responses: An automated non-invasive imaging approach

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Due to climate change and the increasing human population there is a demand for progress in plant breeding. Crop yield is influenced for example by pathogens, insects and other environmental factors. Therefore, it is getting more and more important to select for crop cultivars which can conquer such biotic and abiotic stresses. Currently, a major bottleneck for crop breeding is the analysis of stress tolerance in high throughput. To partially close this gap, we have developed, as part of the 'German Plant Phenotyping Network' (DPPN), a high-throughput platform to analyze plant immunity and disease. Here, we try to understand the biotic stress response, including disease progression and disease resistance in plants. To this end, we established an automated non-invasive high-throughput confocal imaging platform to analyze live plant seedlings under different environmental conditions. Fluorescently marked pathogens and fluorescent dyes will help to find robust phenotypes as candidates for field applications. We have optimized the live tissue imaging platform using *Arabidopsis thaliana* seedlings in interaction with fluorescently labeled *Pseudomonas syringae* pathogenic bacteria and are currently extending our analysis portfolio to include seedlings of crop plants such as barley, wheat, and tomato in interaction with different bacterial and fungal pathogens.

P24 *PHYTOALEXIN DEFICIENT 4* affects physical and chemical properties of wood in hybrid aspen (*Populus tremula* L. x *tremuloides*)

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The function of *PHYTOALEXIN DEFICIENT 4* (*PAD4*) gene in responses to pathogen infection in *Arabidopsis* is well-known, whereas its role in woody plants has not been identified earlier. Therefore, we have selected transgenic hybrid aspen with reduced *PAD4* poplar ortholog expression. The results clearly suggest that down-regulation of *PAD4* affects inhibition of growth and biomass production in *P. tremula* x *tremuloides* in field conditions. However, the wood/cell wall structure and its chemical composition in *PAD4*-deficent trees were improved compared to wild type plants. The wood of *PAD4*-deficient trees may absorb less water, and the structure and composition of hemicelluloses and the cellulose is different in comparison to the wild-type plants. The data obtained suggest that PAD4 in *P. tremula* x *tremuloides* is involved in a growth, wood biomass production and defense responses. However, the detailed role of PAD4 in the cell wall and wood development in *P. tremula* x *tremuloides* requires further studies.
This work was financed by PBS1/A8/16/2013 project.

P25 Identification of a novel receptor-like kinase that is associated with membrane micro-domains and regulates plant immunity

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Emerging evidence indicates the significance of pre-assembled proteins complexes within plasma membrane micro-domains to facilitate an efficient signal transduction during plant–microbe-interactions. Different meso-scale membrane domains (MMDs) are targeted by plant-specific Remorins, which are accepted MMD marker proteins. Remorins have been shown to interact with receptor kinases and other membrane-associated proteins. They may serve as molecular scaffolds and play a role during the protein complex assembly. Here, we used AtREM1.2, a prominent member of this gene family, to identify novel signaling proteins that are putatively involved in plant immunity. One LRR-RLK, we called RICKY1, was specifically interacting with AtREM1.2 in a phosphorylation-dependent manner. RICKY1, a member of the subgroup LRR-VIII-2, is characterized by the presence of an extracellular malectin domain. Using imaging-based interaction studies we confirmed a specific protein interaction in distinct domains at the plasma membrane. Pathogen infection assays revealed an increased susceptibility of *ricky1* mutant lines to *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis.* A closer look at different defense responses showed that the mutant plants are affected in flg22-induced callose deposition, whereas earlier responses are not impaired. These and other data that will be presented indicate that RICKY1 serves as a new component of plant immunity.

P26 A neofunctionalized retrotransposon-derived peptide targets RACB for microtubule depletion at the site of fungal entry

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Plant immunity is overcome by pathogens by the means of effector molecules that manipulate host targets. Most pathogen effectors suppress immunity for successful pathogenesis. Host proteins can also contribute to disease susceptibility if they function in negative control of defense or serve demands of the pathogen. Few examples exist, where targets of pathogen effectors do not function in immunity but more directly support pathogenesis. The parasitic fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*) establishes digitate haustoria in intact epidermal cells of barley. The mechanism of haustoria accommodation is poorly understood but involves the barley susceptibility factor RACB, a small RAC/ROP GTPase. Recent evidence suggests that RACB does not act in negative control of host defense but has a physiological function in polar cell development via regulation of the host cytoskeleton. Additionally, RACB is addressed by an unconventional retrotransposon-derived peptide effector of *Bgh*, which promotes haustoria accommodation and partially co-localizes with RACB at the cell periphery and microtubules. Data suggest that *Bgh* manipulates polar cell host development for entry into differentiated epidermal cells.

P27 Specialization of exocytosis pathways to maintain a stable symbiotic host-microbe interface

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At the heart of the arbuscular mycorrhizal endosymbiosis a specialized host–microbe interface, the arbuscule, is formed which facilitates the exchange of nutrients between plant and fungus. The formation and maintenance of this interface are key steps at which the plant can control the symbiosis. In infected cells multiple exocytosis pathways are operational, marked by distinct SNARE proteins, which may be differentially regulated to control the interface. Phylogenetic analyses allowed the identification of a subset of SNARE proteins that seem dedicated to endosymbiosis. In *Medicago truncatula* these include two related v-SNAREs and one t-SNARE, SYNTAXIN OF PLANTS 13II. These SNAREs are essential to form a symbiotic interface in both AM and rhizobium symbioses. In most dicot plants the *SYP13II* gene, which originated early in the angiosperm lineage, is alternatively spliced resulting in two isoforms, which incorporate different last exons. The ancestral SYP13IIα isoform becomes dominant in cells that form a symbiotic interface where it is essential for the formation of a stable interface. In Medicago the SYP132β isoform plays an essential in root development. Both isoforms show differential labelling of the peri-arbuscular membrane when the arbuscule starts to degenerate; MtSYP132β preferentially labels degrading arbuscules, while the SYP13IIα isoform preferentially marks functional arbuscule fine branches. Our data shows that vesicle traffic to the symbiotic interface is specialized and involves distinct SNARE proteins during its formation and degradation.

P28 Identification of a non-ribosomal peptide that helps *Fusarium graminearum* growing into next cells inside host plant

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The ascomycete fungus *F. graminearum* is a devastating fungal pathogen, can cause yield loss of various cereal crops and contaminates grain with mycotoxins such as zearalenone, fusarin C, deoxynivalenol (DON) and its derivatives which are harmful to health of human and livestock. Using laser microdissection, we profiled *in planta* fungal gene expression during wheat coleoptile infection and identified several virulence genes that are preferentially expressed during invasive growth. Of these genes FG3_54 is an organ-selective NRPSs (Non-Ribosomal Peptide Synthetases) gene cluster, comprises eight co-expressed genes that all required for full virulence on wheat. During infection in wheat coleoptiles, FG3_54 related mutants encountered strong host cell wall deposition, showed low penetration ratio and were blocked in the colonized cells. Identification of a new bANK transcription factor which can induce expression of FG3_54 makes it possible to study the product(s), and the extracellular extracts of FG3_54 induced strain can enhance the virulence of FG3_54 mutants. With HPLC, TLC, LC-MS, GC-MS and NMR analyses, we have identified a peptide supposed to be product of this cluster and could enhance virulence of FG3_54 mutants. Our studies indicate that the product of FG3_54 is a new organ specific pathogenicity factor of *F. graminearum*.

P29 Role of *S*-nitrosoglutathione reductase and nucleoredoxins in redox-mediated plant defence

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In plants, dramatic changes in cellular redox status are observed upon exposure to environmental stresses, including pathogen attack. These changes affect the oxidative status of reactive cysteine thiols in regulatory proteins. To control oxidative protein modifications, plant cells employ the antioxidant enzymes *S*-nitrosoglutathione Reductase 1 (GSNOR1) and members of the Thioredoxin (TRX) superfamily. Immune signalling by the hormone salicylic acid (SA) is particularly dependent on the activity of these enzymes. SA is synthesized in response to challenge by plant pathogens for the establishment of local and systemic immunity. SA accumulation is regulated by cellular levels of *S*-nitrosoglutathione (GSNO), a redox molecule capable of *S*-nitrosylating proteins (*i.e.*, covalent attachment of nitric oxide to cysteines). GSNOR1 is thought to regulate cellular GSNO and global *S*-nitrosylation levels, but it is unknown how GSNOR1 regulates SA biosynthesis. Furthermore, SA recruits the activities of selected TRX enzymes that act as ubiquitous thiol reductases to counteract cysteine oxidation of SA-responsive regulatory proteins, thereby modulating their activities. However, it is unclear how SA controls nuclear redox processes involved in SA-responsive gene activation. Here we show that GSNOR1 regulates SA accumulation by regulating the expression of SA biosynthetic genes and their transcriptional activators. Moreover, we describe Nucleoredoxins (NRX) that represent novel, potentially nuclear localized members of the TRX superfamily. Mutant *nrx1* plants displayed enhanced disease resistance, which was associated with enhanced expression of genes involved in synthesis of salicylic acid. Taken together, the data presented in this thesis demonstrate that GSNOR1 and NRX enzymes play critical roles in regulating synthesis of and signalling by SA in plant immunity.

P30 The development of hyphal symbionts and pathogens relies on fatty acid biosynthesis by the plant host

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Many biotrophic filamentous microbes develop host membrane-surrounded hyphal structures, such as haustoria or arbuscules inside plant cells. The development of these putative feeding structures depends on a genetic program of the host plant that allows compatibility and mediates cellular rearrangement for intracellular accommodation of the hyphal structure. In a forward genetics screen designed to find *Lotus japonicus* mutants that are perturbed in arbuscular mycorrhiza development we identified the mutant *disorganized arbuscules* (*dis*) that is impaired in arbuscule branching. We combined classical mapping with next generation sequencing to identify the causative mutation. The *DIS* gene, encodes an enzyme involved in fatty acid elongation, of which three paralogs are present in the *Lotus japonicus* genome. Promoter-*GUS* localization showed that the *DIS* promoter is active in arbuscule containing cells. A DIS-YFP-fusion was targeted to plastids, consistent with the subcellular localization of fatty acid biosynthesis. The simultaneous presence of KASI gene related to housekeeping function and a symbiosis-related paralog (DIS) is conserved in AM-forming dicotyledons. In contrast, the genomes of eight plant species, which are unable to form AM, contain only a single KASI gene. Interestingly, a *kasI* mutant of *Arabidopsis thaliana* impairs development and reproductive success of the biotrophic pathogens *Hyaloperonospora arabidopsidis* (oomycete) and *Erysiphe cruciferarum* (ascomycete). Taken together these data indicate that host fatty acid biosynthesis might be a critical compatibility factor for mutualistic and parasitic interactions of plants with biotrophic filamentous microbes.

P31 Function and regulation of endomembrane trafficking in stomatal responses and its role in plant immunity

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The FLAGELLIN SENSING 2 (FLS2) receptor kinase mediates immunity against bacterial infections through the perception of flagellin (flg22). Activated FLS2 receptors are removed from the plasma membrane and internalized into the lumen of multi-vesicular bodies for vacuolar degradation. Arabidopsis mutants affected in key regulators of endomembrane trafficking are impaired in their defence against bacterial infection, some of them particularly showing defects in microbe-induced but not abiotic stress-induced stomatal closure. This suggests a specific function of membrane trafficking in stomatal closure upon biotic stress.
To address how PAMP-induced endomembrane transport translates into guard cell responses, we have performed a reverse genetic screen and identified mutants that were specifically impaired in stomatal closure upon flg22 treatment. Here, we will report results on the role of a small Rab GTPase, regulating late endosomal-to-vacuole trafficking, in stomatal responses. Mutant plants are compromised in microbe-induced stomatal closure which correlates with an enhanced susceptibility to bacterial infection. We are currently investigating if the mutant stomatal phenotype results from a specific role of the affected Rab GTPase in the transport of activated FLS2 to the vacuole or whether it has a more general impact on subcellular trafficking.

P32 *mlo*-mediated powdery mildew resistance of Arabidopsis coincides with differentially altered MAMP-triggered responses

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Mutation of distinct members of the Mildew Resistance Locus O (MLO) family renders plants resistant against penetration by biotrophic powdery mildew fungi on a single cell basis. Localization of barley MLO at the plant plasma membrane and recruitment to fungal penetration sites during pathogen attack points to a role of the protein in local suppression of plant immunity. Although the protective effect of *mlo* has long been known, the functioning of MLOs in suppression of cellular defense remains largely elusive. Arabidopsis *mlo2 mlo6 mlo12* mutants exhibit complete resistance against compatible powdery mildew fungi. Requirement of non-host resistance components for *mlo*-mediated immunity and co-expression of *MLO*s with basal defense genes suggests a contribution of pattern-triggered immunity mechanisms to MLO function. In our aim to decipher the impact of MLO proteins on cellular defense we investigated microbe-associated molecular pattern (MAMP)-triggered responses of Arabidopsis *mlo* mutants. Strikingly, we found that while mutation of *mlo2 mlo6 mlo12* results in reduced formation of reactive oxygen species (ROS) specifically in response to chitin, phosphorylation of the mitogen-activated protein kinases (MAPKs) MPK3/MPK6 occurs faster and is prolonged after both, chitin and flg22 treatment. This indicates differential contribution of MLO proteins to distinct signaling pathways. Consequently, we examine the requirement of intact ROS and MAPK signaling for *mlo*-mediated resistance. Additional investigation of MAMP-triggered calcium fluxes and stomatal closure in *mlo* mutants will further advance our knowledge of MLOs' interference with cellular defense signaling.

P33 Improving nutritional quality and fungal tolerance in soybean by expressing an oxalate decarboxylase from *Flammulina velutipes.*

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Soybean (*Glycine max*) seeds are important sources of dietary proteins; however, they also contain anti-nutritional metabolite oxalic acid (OA). Excess dietary intake of OA leads to nephrolithiasis due to the formation of calcium oxalate crystals in kidneys. Besides, OA is considered as a pathogenesis factor for the devastating phytopathogenic fungus *Sclerotinia scleriotorum* which synthesizes and secretes OA during host colonization. Here, we report reduction of OA level in transgenic soybean (upto 73%) seeds by constitutive expression of an oxalate degrading enzyme, oxalate decarboxylase (FvOXDC) of *Flammulina velutipes.* Reduced OA content was interrelated with the associated increase in seeds micronutrients such as calcium, iron, and zinc. Moreover, constitutive expression of *FvOXDC* led to improved tolerance to the fungal pathogen *Sclerotinia sclerotiorum.* Importantly, *FvOXDC*-expressing soybean and grass pea plants were similar to the wild type with respect to the morphology and photosynthetic rates. Taken together, these results demonstrated improved seed quality and tolerance to the fungal pathogen in soybean, by the expression of an oxalate degrading enzyme.

P34 Cell-to-neighboring-cell signaling in plant immunity

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Pathogen perception by a plant cell triggers the rapid activation of cell-autonomous defenses aimed at halting the infection. Subsequently, a signal is send to neighboring cells, which prime their defenses to be prepared for an attack. Components that might act in this process are reactive oxygen species, calcium and calcium-dependent protein kinases. My aim is to establish the mechanistic and molecular basis underlying communication between cells in plant immunity. To perform these studies, we have set-up a system to follow cell-to-cell signaling at the single cell level. For this purpose we use chimeric plant tissues expressing the Yellow Cameleon 3.6 biosensor. In these chimeric tissues we can identify cells that are either wildtype or mutated for specific pattern recognition receptors. This system allows us to compare MAMP-triggered, cell-autonomous defense activation and rapid defense priming responses in neighboring cells. Our initial observations confirm that a microbe-associated molecular pattern (MAMP)-induced Ca^{2+} influx wave propagates from an initial pathogen-perceiving cell to neighboring cells. This approach enables us to study and identify new components involved in cell-to-cell signaling.

P35 We're all different! Functional diversification of the HopF family of type III secreted effectors

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By injecting an arsenal of virulence proteins called type III secreted effectors, the bacterial pathogen *Pseudomonas syringae* can cause outbreaks on a wide variety of plant species. One such family of effectors is the HopF family which now includes over 200 members found from a diverse number of *P. syringae* strains that are virulent on herbaceous and woody plants alike. However, research has focused solely on two members of this family: HopF1 from the bean pathogen *P. syringae* pathovar *phaseolicola* 1449B and HopF2 from the *Arabidopsis /* tomato pathogen *P. syringae* pathovar *tomato* DC3000. While both of these effectors play a key role in determining virulence or immunity in their respective plant hosts, the functions of the rest of the HopF family remains uncharacterized. With the HopF family split into a minimum of 6 distinct clades, this family is abundant in genetic diversity. Using *P. syringae* pathogenicity assays, we have uncovered novel functions for multiple members of the HopF family in *Arabidopsis*. The genetic requirements of these interactions are being determined and highlight the functional diversity that exists within the HopF family as well as the host resistance mechanisms that contend with them.

P36 An assay for entry of pathogen effectors into host cells?

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A successful colonization of plants by prokaryotic and eukaryotic pathogens requires active effector-mediated suppression of defense responses and host tissue reprogramming. Secreted effector proteins can either display their activity in the apoplast or translocate to host cells and affect plant functions there. While bacterial pathogens use Type III Secretion System (T3SS) for injecting effectors into plant cells the molecular mechanisms of effector delivery by eukaryotic phytopathogens remain elusive. Here we report the establishment of an uptake assay that is based on the ability of a bacterial biotin ligase, BirA, to biotinylate *in vivo* all proteins that carry a small peptide, the Avitag, which serves as substrate for the enzyme. The assay relies on the stable expression of BirA in the cytoplasm of the host plant and the engineering of phytopathogen effectors with the Avitag. The only effectors to be biotinylated by BirA are the ones that translocate into the host cell. Hence biotinylation becomes a hallmark of uptake. We have initially established the feasibility of the assay in a transient expression assay in. *Nicotiana benthamiana.* Currently we are transferring the assay to the *Ustilago maydis*–maize pathosystem and we will present and discuss here our first results.

P37 *Rhizobium leguminosarum* Norway infects *Lotus* via "crack-entry"

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Legumes can overcome nitrogen limitation by associating symbiotically with nitrogen-fixing rhizobia. Infection is often initiated by the inward growth of infection threads (ITs). These structures guide rhizobia towards the root inner cortex, where they intracellularly colonize it [1]. An alternative infection mechanism called "crack-entry" exists. This is believed to represent a more ancient infection mode, in which bacteria enter through physical cracks at the root epidermis[2].
IT formation is the predominant mode of infection in *Lotus*. However, we have identified an environmental isolate, *R. leguminosarum* Norway that intracellularly colonizes *L. burttii* by "crack-entry". To explore the natural diversity of this infection we inoculated more than 30 different *L. japonicus* ecotypes with a GFP-tagged *Rl* Norway strain and phenotyped infection using epi-fluorescence and confocal microscopy. In all ecotypes, *Rl* Norway penetrated via "crack-entry". However, we observed a variety of nodulation phenotypes that we classified into 3 groups: i) no infection/nodules, ii) bumps and tumours and iii) white nodules. To identify *Rl* Norway genes involved in controlling "crack-entry" infection, we sequenced the genome of *Rl* Norway and started generating deletion mutants of genes involved in nod factor synthesis, exopolysaccharide production and effectors secretion. Herewith we aim to understand this poorly studied infection mode.

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2. Held *et al*. (2010) Common and not so common symbiotic entry. *Trends Plant Science* **10**: 540–545.

P38 A novel family of symbiosis-associated arabinogalactan peptides is differentially expressed in *Medicago truncatula* in response to bacterial and fungal symbiosis

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Small post-translationally modified peptides have emerged as key signaling molecules in plants that regulate cell-type identity and organogenesis through autonomous and non-autonomous cell–cell signaling pathways. A number of these peptide hormones, namely Enod40, CLE, and NCR peptides, have previously been identified as regulators of nodule development, differentiation, and nodule number in rhizobium–legume symbiosis – highlighting the importance of these signaling molecules in plant–microbe interactions. Here, we have identified a novel three member family of symbiosis-associated arabinogalactan peptides (SAPs) in *Medicago truncatula* that are differentially expressed in roots colonized by either arbuscular mycorrhizal fungi or nodulated with *Sinorhizobium meliloti*. Arabinogalactan proteins represent a highly diverse group of cell wall glycoproteins that have been previously linked to plant cell differentiation and implicated in other root–microbe interactions. Knockdown of SAP3 using RNAi in transgenic hairy roots baited with *S. meliloti* result in white stunted nodules and chlorotic leaves, indicating a loss of nitrogen fixation. Furthermore, SAP3 RNAi nodules have aberrant cellular anatomy and fewer cells harboring mature bacteroids. After proteolytic processing of predicted secretion and GPI-anchor signals, the mature SAP peptides are expected to be between 14–28 amino acids in length and therefore may act as putative peptide ligands.

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P39 Effects of silicon supplementation on some phenolics (HPLC evaluation) of strawberry (*Fragaria × ananassa var. Selva*) plants in vegetative stage

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Silicon (Si) application could influence phenolic and terpenoid compound metabolism. In this work the effects of Si supplementation (3 mM, as Na_2SiO_3) were studied in vegetative stage of strawberry (*Fragaria × ananassa var. Selva*) plants. Plants were grown in pot, irrigated %85 field capacity and cultivated for 6 weeks under greenhouse conditions. Plant dry matter production, protein content and total phenols (free and bond) increased in Si-applied plants but soluble carbohydrates, total starch and free amino acids were decreased in the leaves. Application of Si significantly increased phenylalanine ammonia-lyase (PAL) and decreased polyphenol oxidase (PPO) activities. Soluble peroxidases (SP), ionically (IBP) and covalently bound peroxidases (CBP) activities were not affected by Si treatment. HPLC evaluation of some phenolics in the shoot and root has revealed that phenolic profile has changed in +Si treated plants compared with –Si ones. Bonded caffeic acid, ellagic acid, soluble gallic acid, epicatechin and quercetin were increased and bonded epicatechin and chlorogenic acid, soluble ellagic acid and kaempferol were decreased in the shoot. Bonded gallic acid, caffeic acid, chlorgenic acid and ellagic acid and soluble epicatechin were increased but bonded p-coumaric acid was decreased in the roots. Our results suggest Si role in enhancing strawberry growth and phenols metabolism.

P40 Transcriptional co-regulation between ER bodies and indole glucosinolate metabolism, a potential strategy to maximize the efficiency of defensive traits

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Plant secondary metabolites in many cases comprise a layer of defense response. Plants of the order Brassicales accumulate a class of sulfur-containing metabolites named glucosinolates, which exhibits anti-pathogenic activity against wide range of potential enemies including herbivorous insects, fungi, and bacteria, when activated by specific enzymes called myrosinases. Recently it has been shown that indole glucosinolates are not only required for defense response but also for establishing a beneficial interaction with soil-borne fungi. However, the spatiotemporal control of glucosinolate activation remains unclear. Here we report an identification of a novel class of myrosinases that arose independently from the historically proven myrosinases (THIOGLUCOSIDE GLUCOHYDROLASEs, TGGs). One of the novel myrosinases, PYK10/BGLU23, is highly enriched in the ER bodies, a Brassicaceae-specific intracellular compartment. Interestingly, *PYK10*, the genes required for ER body formation, and the genes encoding proteins that interact with PYK10 are strongly co-expressed with indole glucosinolate biosynthetic genes, which are evidenced by a large-scale and unbiased transcriptomic dataset (> 10,000 microarray experiments). The co-expressed cluster exclusively contained these genes, amongst many other genes that either encode homologous proteins or show similar tissue-specific expression patterns. Our results strongly indicate that ER body system and indole glucosinolate metabolism is functionally coordinated in *A. thaliana* to maximize the efficiency of the overall pathway. We hypothesize that the intracellular discrimination of indole glucosinolates and PYK10 myrosinase by developing ER bodies has a critical role in establishing a beneficial interaction with surrounding fungi in natural soil.

P41 Genotype by genotype dependence of hypovirus induced stress in chestnut blight fungus *Cryphonectria parasitica*

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Cryphonectria hypovirus 1 (CHV1), a virus conferring reduced virulence to chestnut blight fungus *Cryphonectria parasitica*, has been successfully used as a biocontrol agent. Several subtypes of the hypovirus have been isolated in Europe, having the impact on the fungus from mild to severe. It has been shown that hypovirus-infected fungal strains had a number of differentially expressed genes compared to the virus-free strains, among which were genes included in general stress response of the fungus. The objective of the study was to reveal whether the virus stress induction in fungus plays major role in decreasing fungal virulence. Various virus strains, previously characterised as mild or severe, were transferred to different Croatian isolates of *C. parasitica* and the activity of the stress enzymes catalase, glutathione S-transferase and superoxide dismutase was measured. Virus induced change in fungal stress enzymes activity, but the intensity and direction of the impact was dependent on virus strain and virus–host combination.

P42 Genetic transformation of Colombian isolates of *Phytophthora palmivora* with fluorescent proteins for histological characterization of oil palm bud rot disease

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Bud rot (BR) is the most devastating disease affecting oil palm (*Elaeis guineensis*) crops in Colombia. Its causal agent, *Phytophthora palmivora*, is a hemibiotrophic pathogen that starts the infection of young oil palm leaves with a small lesion, allowing the colonization of opportunistic pathogens, causing several damages or plant death. In order to characterize the initial stage of BR, we transformed two isolates of *P. palmivora* to include the fluorescent proteins CFP-SKL, eGFP and mRFP1 for histological visualization using confocal microscopy. Stable transformants were obtained using *A. tumefaciens* mediated transformation; these transformants were evaluated using PCR, RT-qPCR and Southern Blot. Some transformants were observed with confocal microscopy to detect which one has the highest fluorescence. Transformed colonies with eGFP showed the best fluorescence and some of these colonies showed normal growth in petri dishes and similar virulence in green apple compared to de untransformed isolate, so these transformants may be suitable to start the characterization of the infection process. The aim of this research is to improve the characterization of the initial stages of the disease, towards the development of methodologies that allows the early identification of resistant or susceptible oil palm materials to BR.

P43 Identification and characterization of *in planta*-expressed secreted effector proteins in *Ustilago hordei*

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Research over the past decades has shown that plant pathogen effectors determine the outcome of host–pathogen interactions. Since effectors play essential roles in virulence of plant pathogens, their identification and functional characterization can provide new insights into the molecular basis of host–pathogen interactions and can eventually provide potential novel targets for improving current disease control strategies. In this study, in order to understand how *Ustilago hordei* establish disease in barley plant we performed robust microarray analysis in *U. hordei* infected barley. To identify genes involved in pathogenicity of *U. hordei*, we compared the transcriptome of different time points of infected barley to *in vitro* growing *U. hordei*. In total, 170 candidate secreted effector proteins (CSEPs) encoding genes were differentially up-regulated at least one of these time points. For further characterization of CSEPs, 18 highly up-regulated genes were knocked-out in *U. hordei* to assess their contribution in virulence. Virulence assay performed for mutants revealed 9 CSEPs that are required for full virulence of *U. hordei*. In addition, Y2H and pull-down assays were performed for the three of CSEPs that contribute virulence to identify their respective plant targets.

P44 *At*Pep1 and its plasma membrane receptors are internalized as a complex via clathrin-mediated endocytosis

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The small signaling peptide, the plant elicitor peptide1 (Pep1) is expressed upon biotic stresses and induces innate immune responses. The *Arabidopsis thaliana* Pep1 (*At*PEP1) binds the plasma membrane receptors PEPR1 and PEPR2 with high affinity. Although a large amount of information about the components and responses triggered by *At*Pep1 is available, its subcellular dynamics remains largely unknown. In the present study, we developed a bioactive fluorescently labeled *At*Pep1 to study its behavior in living cells. We found that the labeled *At*Pep1 was able to bind the plasma membrane very quickly in a receptor-dependent manner. Subsequently the receptor–ligand complex was internalized via clathrin-mediated endocytosis and trafficked to the lytic vacuole, passing through early and late endosomal compartments. Impairment of *At*Pep1/PEPRs complex internalization compromised the innate immune responses. Our findings provide the in vivo visualization of the signaling peptide *At*PEP1 in living plant cells, thus giving new insights on its intracellular fate and dynamics, and also serve as an excellent model to study the implications of endocytosis in plant immunity.

P45 A novel pathosystem between *Phytophthora* and the moss *Physcomitrella patens* as a model for studying cellular plant defence responses

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In general, live cell imaging of plant–pathogen interactions is hampered by the tissue complexity and multi-cell layered nature of the host. Therefore, we developed a novel *Phytophthora* pathosystem with *Physcomitrella patens* as host. The single-cell layered protonema of this moss is ideal for visualizing interactions with the pathogen over time using advanced fluorescence microscopy.
Of four tested *Phytophthora* species, *Phytophthora infestans* and *Phytophthora capsici* were able to successfully penetrate moss cells and showed invasive hyphal growth and sporangia formation on moss tissue. Upon infection, several defence-related genes of *Physcomitrella patens* were upregulated and local cell death was induced. At a cellular level, we observed repositioning of the nucleus, accumulation of cytoplasm and rearrangement of the actin cytoskeleton. Furthermore, penetration of *Phytophthora* was often blocked by the deposition of cell wall material in papilla-like structures, which is also a common defence response observed in higher plants.
We will exploit this novel pathosystem to obtain new insights in the regulation of cellular defence responses, e.g. the molecular pathways that regulate targeted exocytosis towards the site of pathogen attack.

P46 Barley *Ror1*, a component required for broad-spectrum powdery mildew resistance, encodes an actin-binding protein

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Loss-of-function alleles of the barley *Mlo* locus confer durable broad-spectrum resistance against the powdery mildew disease caused by the ascomycete *Blumeria graminis* f.sp. *hordei*. The molecular mechanisms underlying this unusually robust type of plant resistance remain largely unexplored. We report the cloning of the barley *Required for mlo-specified resistance 1* (*Ror1*) gene, which was identified in a genetic suppressor screen as a component required for full *mlo*-based resistance. Previously, we mapped *Ror1* to a region of the long arm of barley chromosome 1 that exhibits low frequencies of recombination and a perturbed gene order in comparison to other grass species. The combination of updated physical contigs in the barley genome assembly at the *Ror1* locus and whole transcriptome shotgun sequencing (RNAseq) led to the identification of *Ror1*. The gene encodes an actin-binding protein, indicating that *mlo* resistance relies on intracellular transport processes. Notably, genetic data suggest that the Ror1 defense pathway is distinct from the pathway relying on the Ror2 soluble *N-ethylmaleimide*-sensitive-factor attachment receptor (SNARE) protein, which is believed to contribute to vesicle fusion events at the plasma membrane. Ror1 thus defines a separate route required for antifungal broad-spectrum immunity in barley.

P47 Monitoring subcellular dynamics of effectors delivered from *Pseudomonas* T3SS into plant cells

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Various phytopathogens deliver effectors to reprogram host cells that facilitate their pathogenesis. The gram-negative bacteria such as *Pseudomonas* uses type III secretion system (T3SS) to deliver effectors into host cells. However, very little is known about dynamics and subcellular action of effectors when delivered directly from pathogen because tagging effectors with fluorescent proteins such as GFP hinder their delivery through the T3SS. To overcome this, we have optimized split GFP method using super folder GFP (sfGFP) to monitor the *Pseudomonas* effectors delivered through the T3SS. We will discuss our results on characterizing candidate *Pseudomonas* effectors dynamic localization in two model plants, *N. benthamiana* and *Arabidopsis thaliana*. *Pseudomonas* has been known to inject more than 20 effector proteins into host cells through the T3SS. However, only few effectors have been studied about their contribution on bacterial pathogenesis. Subcellular localization information about these effectors might insight their roles to contribute the bacterial pathogenesis. The system can facilitate better understanding of dynamic localization of effectors during host–microbe interaction.

P48 A timely secretion of fungal monooxygenase blocks plant innate immunity

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Jasmonic acid (JA) is a plant growth and immune response regulator whose activity is fine-tuned by its distinct derivatives. While hydroxylation to 12-hydroxyjasmonic acid (12OH-JA) is a plant mechanism to inactivate JA signaling, it serves as a tuber-inducing factor in potato. Despite its versatility, biosynthesis of 12OH-JA was elusive thus far. In a model plant pathosystem, we found that the rice-blast fungus *Magnaporthe oryzae* produces a monooxygenase (Abm) that converts endogenous JA into 12OH-JA. It is incredible that the fungal pathogen produces a phytohormone (JA) and secretes a tailored derivative (12OH-JA) of it to suppress plant innate immunity. In addition, the fungal pathogen secretes the monooxygenase Abm in a timely manner i.e. only during invasive growth likely to convert plant JA into 12OH-JA to further aid host colonization. Interestingly, loss of Abm function leads to accumulation of fungal methyl JA (MeJA), which activates the plant defense response and blocks invasion by the blast fungus. This study not only sheds light on the chemical arms race during plant–pathogen interaction, but also provides Abm as an antifungal target and outlines a synthetic strategy for transformation of a versatile small-molecule phytohormone.

P49 Identifying the protein and RNA interactors of BEC1054

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Blumeria graminis f. sp. hordei is an economically important biotrophic fungal pathogen which causes powdery mildew disease. The fungus compromises host immunity through the delivery of effectors at the haustorial complex. One such effector, *Blumeria* Effector Candidate 1054 (BEC1054) is predicted to be a ribonuclease-like protein, and has had its effector function verified (Pliego *et al.,* 2013). A recombinant BEC1054 protein with an N-terminal His-tag was expressed in *E. coli*, purified, and used as the bait for *in vitro* liquid chromatography mass spectrometry. A total of 247 putative interacting proteins were identified which interacted solely with BEC1054. Small 40S ribosomal subunit proteins and eukaryotic elongation factors (eEF)s were significantly overrepresented within this dataset. Five proteins were validated through a yeast-two-hybrid approach: 1) ribosomal subunit 40S 16 protein; 2) elongation factor 1 gamma; 3) PR5 protein; 4) malate dehydrogenase and 5) glutathione-S-transferase (GST). Bimolecular Fluorescence Complementation has been used to validate two of these (PR5 and GST) and one other in the host plant barley. We hypothesise that BEC1054 interacts with the ribosome, outcompeting Ribosome Inactivating Proteins (RIP)s and helping to prevent the infected cell's death. This allows this biotrophic fungus to continue feeding from the host cell.

P50 Members of the *Phytophthora infestans* PexRD12 effector family target host endomembrane compartments and exosomes

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Filamentous plant parasites engage in intimate contact with host cells through specialized infection structures called haustoria. In plant cells haustoriated by the Irish potato famine pathogen *Phytophthora infestans*, endomembrane trafficking pathways are perturbed. We hypothesize that *P. infestans* delivers effector proteins into host cells to manipulate endomembrane trafficking. Recent *in planta* screens of RXLR effectors revealed the PexRD12 effector family, whose members accumulate in endomembrane compartments and modify the number of FYVE-labeled vesicles in plant cells. We report that members of the PexRD12 family accumulate not only in distinct endomembrane compartments but also in exosomes. During *P. infestans* infection, PexRD12 family members label the extra-haustorial membrane as well as vesicles that accumulate around haustoria. The identification of host proteins associated with PexRD12 family members is ongoing, and should help reveal the mechanism by which *P. infestans* manipulates host vesicular trafficking.

P51 *Lotus japonicus* RAM1 is required for arbuscule development

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Mineral nutrients such as phosphate and nitrogen are limiting factors for plant growth and development. Arbuscular mycorrhiza (AM) symbiosis is the most widespread strategy of plants to acquire these nutrients and most agronomically important crop species are able to form AM symbiosis. Root colonization by AM fungi culminates in the formation of highly branched hyphal structures called arbuscules, which release the mineral nutrients to the plant (Gutjahr and Parniske, 2013). The amount and branching status of arbuscules might influence symbiotic nutrient-transfer efficiency. However, the regulation of arbuscule branching is poorly understood. To identify molecular players in arbuscule development a forward genetic screen was performed and identified the *Lotus japonicus* mutant *reduced and degenerate arbuscules* (*red*), which is impaired in arbuscule branching. A causative EMS mutation was found in the *red* mutant perturbing a gene encoding the GRAS protein REQUIRED FOR ARBUSCULAR MYCORRHIZA 1 (RAM1). Our study provides insights into the transcriptional regulation and role of *RAM1*.

P52 Orchids utilize trehalose from mycorrhizal fungi by the action of trehalase

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During early stages of development, orchid nutrition depends exclusively on mycorrhiza. The question remains, however, which compounds are transferred in mycorrhizas and what are their roles.

To address the question, we have tested the ability of *Dactylorhiza majalis* protocorms to utilize different saccharides under *in vitro* conditions. The results confirmed sucrose, fructose, glucose, raffinose, sorbitol and trehalose as suitable compounds to provide energy and carbon skeletons for protocorm growth. The ability to utilize trehalose as the sole energy source is unique among plants.

In our experiments, the application of trehalase-specific inhibitor, validamycin A, to trehalose-supported asymbiotic cultures led to nearly total inhibition of protocorm growth which indicates trehalase activity being responsible for trehalose utilization in orchids. Semiquantitative RT-PCR revealed high expression levels of trehalase in trehalose-supported asymbiotic tissues and similarly in mycorrhizal plant tissue. These data strongly support trehalose role in sugar transport from fungus to the orchid.

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P53 Identification of a novel organogenesis suppressor that allows symbiotic dualism in legumes

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In contrast to most other land plants, legumes gained the ability to simultaneously host two symbioses: the widespread arbuscular mycorrhiza (AM) and the legume-specific root nodule symbiosis (RNS). Intriguingly, the initial signal transduction upon microbial perception is funneled through the same common symbiosis pathway (CSP). However the morphological consequences differ significantly. While an entire nodule organogenesis program is initiated during RNS to host rhizobia, the AM fungus colonizes existing inner root cortical cells. This raises the ultimate question how the host plant discriminates between both symbionts downstream of CSP activation. We identified MYCREM, a member of the remorin protein family, which strictly evolved in legumes that undergo this symbiotic dualism. We show that *mycrem* mutants are altered in a regulatory system that spatially suppresses inner cortical cell divisions in the presence of both symbionts. Consequently these mutants not only develop more nodules under these conditions but also display a lateral root phenotype. In addition we have identified components that contribute to the activation of the *MYCREM* promoter that provide insights into the complexity of this novel regulatory circuit that is required under natural conditions.

P54 Avr4 induces Cf-4 receptor-like protein association with the BAK1/SERK3 receptor-like kinase to initiate receptor endocytosis and plant immunity

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The first layer of plant immunity is activated by cell surface receptor-like kinases (RLKs) and proteins (RLPs) detecting infectious pathogens. Constitutive interaction with the RLK SUPPRESSOR OF BIR1 (SOBIR1) contributes to RLP stability and kinase activity. As RLK activation requires trans-phosphorylation with a second associated RLK, it remains elusive how RLPs initiate downstream signaling. To address this, we investigated functioning of Cf RLPs mediating immunity of tomato against *Cladosporium fulvum*. We employed live-cell imaging, gene silencing and co-immuno-precipitation in tomato and *Nicotiana benthamiana* to investigate the requirement of associated kinases for Cf activity and ligand-induced subcellular trafficking of Cf-4. Upon elicitation with matching effector ligands Avr4 and Avr9, BRI1-ASSOCIATED KINASE 1 (BAK1/SERK3) associates with Cf-4 and Cf-9. Furthermore, Cf-4 that interacts with SOBIR1 at the plasma membrane, is recruited to late endosomes after elicitation. Significantly, BAK1/SERK3 is required for Avr4-triggered endocytosis of Cf-4, effector-triggered defenses, hypersensitive response and tomato resistance against *C. fulvum*. Our observations indicate RLP-mediated immune signaling, resistance and endocytosis require ligand-induced recruitment of BAK1/SERK3, reminiscent of BAK1/SERK3 interaction and subcellular fate of the FLAGELLIN SENSING 2 RLK. This reveals that diverse classes of cell surface immune receptors share common requirements for signaling initiation of resistance and endocytosis.

P55 Whole-genome consequences of a novel transcription factor acquisition

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Gene duplication is believed to be an important source of new genes. At least 50% of prokaryotic genes and over 90% of eukaryotic genes are estimated to have originated through gene duplication. Duplicated genes often diverge and evolve new functions. The emergence of new genes with novel functions in many cases requires reprogramming of the regulatory networks to ensure that the new paralogs are properly expressed. Such network rewiring is especially important when transcription factors are duplicated, because this may have detrimental fitness effects through paralog interference or gene dosage. Several studies have investigated the regulatory divergence between paralogs on a genome-wide level. Together, these studies show that such changes in gene regulation occur frequently and are important drivers of functional and morphological evolution. However, despite the importance of the evolution and divergence of paralogous gene regulation, the exact molecular mechanisms and mutational pathways that lead to the emergence of such novel regulatory networks remain largely unknown. To investigate these mechanisms we have chosen a perfect model system: a novel plant transcriptional regulator, which has recently emerged via duplication. This regulator has also acquired a new function and now orchestrates a completely novel developmental program not present in the ancestor. We aim to decipher the molecular details behind the evolution of this transcription factor from a completely redundant gene copy to a present day key plant regulator. Together these results will lead to a better understanding of molecular mechanisms behind the emergence of novel regulatory networks.

P56 Subcellular localization reveals new targets and functions for *Colletotrichum higginsianum* effectors

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Colletotrichum higginsianum causes anthracnose disease on cruciferous plants, including *Arabidopsis*. It uses a hemibiotrophic infection strategy, involving formation of several specialized cell types. After appressoria puncture host surfaces, bulbous biotrophic hyphae develop inside living host cells, surrounded by a modified host plasma membrane; finally, the fungus switches to destructive necrotrophy, associated with thin filamentous hyphae. The *C. higginsianum* genome encodes 365 putative secreted effectors (ChECs) having an N-terminal secretion signal and no homology to proteins outside the genus *Colletotrichum*. Deep transcriptome sequencing revealed a set of 97 plant-induced ChECs, of which 67 were highly expressed in appressoria and/or biotrophic hyphae. Important clues to effector targets and functions may come from knowing their destination inside plant cells. We therefore transiently expressed these ChECs as N-terminal fusions with GFP in *Nicotiana benthamiana* leaf cells for confocal microscopy. Most proteins (38) were distributed between the plant cytosol and nucleus, similar to GFP alone. However, 11 targeted the plant nucleus, including 6 labelling nucleoli or Cajal bodies, 12 labelled organelles, including Golgi (1) and peroxisomes (3), and one decorated microtubules. These specific localization patterns suggest some ChECs are translocated into plant cells during infection. Progress towards functional characterization of selected ChECs will be presented.

P57 Guard cell SLAC1-type anion channels mediate flagellin-induced stomatal closure

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Upon recognition of the bacterial elicitor flg22, stomatal pores in the leaf surface close, which hinders the entry of microbes. The molecular mechanism of this response and its interrelation with ABA signaling, were studied with the non-invasive nano-infusion technique and microelectrodes. Voltage clamp experiments with double barreled electrodes revealed that flg22 stimulates both the SLAC1 and SLAH3 anion channels in guard cells. Loss of both channels resulted in guard cells that lack flg22-induced anion channel activity and stomata that did not close in response to flg22 and ABA. Because of the similarities in stomatal responses to flg22 and ABA, we studied mutants of both signaling pathways. Rapid flg22-dependent stomatal closure was impaired in plants that were flagellin receptor (FLS2) deficient, as well as in the *ost1-2* (Open Stomata 1) mutant, which lacks a key ABA-signaling protein kinase. In contrast, stomata of the ABA protein phosphatase mutant *abi1-1* (ABscisic acid Insensitive 1) remained flg22 responsive. These results suggest that the initial steps in flg22- and ABA signaling are different, but the pathways merge at the level of OST1 and lead to activation of SLAC1 and SLAH3 anion channels.

P58 A pioneering imaging approach to arbuscule development in rice

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Within the roots of most land plants beneficial arbuscular mycorrhiza fungi (AMF) form complex tree-shaped feeding structures called arbuscules. Monumental cellular re-differentiation and reprogramming in the inner root cortex result in the *de novo* synthesis of a host-derived membrane that surrounds the arbuscule, the peri-arbuscular membrane (PAM) [1]. This functional symbiosome interface facilitates nutrient exchange between fungus and plant. The extent to which fungal and host membranes are tailored for this plant–fungal dialogue and our understanding of how different PAM-specific proteins are retained within the PAM remain unknown. This is partly due to the low resolution of live-cell imaging of inner cortical rice root cell layers using conventional confocal laser scanning microscopy (CLSM). The aim of this project was to 1.) develop innovative imaging approaches to investigate ultra-structural modifications to plant and fungal membranes during arbuscule differentiation and 2.) to pioneer high resolution live-cell bioimaging to investigate dynamic changes to PAM-specific proteins during arbuscule development. High pressure frozen (HPF) Transmission Electron Microscopy (TEM) of rice roots colonized by *Rhizophagus irregularis* revealed novel membrane structures at the AMF-plant interface. Deep-tissue whole mount imaging using multi-photon confocal microscopy (MPCM) showed that protein accumulation at the PAM is temporal and within distinct subdomains. Our data suggests that during symbiosis PAM-specific protein localization at the plant fungal interface is under spacio-temporal control and maintained in a highly dynamic manner.

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P59 Structural basis of pathogen recognition by a novel integrated HMA domain in a plant NLR immune receptor

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Rice blast, caused by the fungal pathogen *Magnaporthe oryzae*, is one of the most economically devastating diseases worldwide. Approaches to controlling blast disease have mainly been *via* the deployment of NLRs. Here we describe the structural basis for direct recognition of AVR-PikD, an effector from *M. oryzae*, by the rice intracellular NLR Pikp comprising of a pair of proteins Pikp-1 and Pikp-2. Recognition of AVR-PikD by the Pikp is mediated by AVR-PikD binding to a heavy metal associated (HMA) domain of Pikp-1 (Pikp-HMA). X-ray crystallography of the Pikp-HMA/AVR-PikD complex revealed that Pikp-HMA makes a dimer and adopts ferredoxin fold with no metal biding. AVR-PikD binds to one monomer of the Pikp-HMA dimer. In the Pikp-HMA/AVR-PikD complex, the AVR-PikD^{his46} side chain is buried within a pocket on the Pikp-HMA surface and contributes to hydrogen bonds/salt bridge interactions. Point mutations in AVR-PikD^{46} as well as in other possible biding sites AVR-PikD^{64} and AVR-PikD^{66} disrupted Pikp-HMA/AVR-PikD interaction. It is notable that the ancestral allele AVR-PikD has His at the position 46 but the other AVR-Pik alleles have Asn and evade recognition by Pikp.

P60 Identification and characterization of *Golovinomyces cichoracearum e*ffectors

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G. cichoracearum, the causative agent of squash powdery mildew, is an obligate biotrophic plant pathogen. Through use of the host-induced gene silencing technique, genetic manipulation of this previously-untransformable fungus is now a possibility. Fifty candidate effector genes were identified, cloned and screened in the model *G. cichoracearum* host *Arabidopsis thaliana.* Effectors exhibiting an infection phenotype when silenced were selected for further molecular and cellular characterization, including localization and identification of protein targets in the infected host cell. These experiments will shed light on the mechanism of infection in this biologically interesting and largely unknown fungal system.

P61 A novel *in vitro* infection system to study *Phytophthora*-host interactions

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One of the most devastating plant diseases worldwide is late blight on potato and tomato caused by the oomycete pathogen *Phytophthora infestans*. During the early biotrophic phase of infection, *Phytophthora* penetrates host tissue and thereafter forms specialized feeding structures called haustoria. Here, effectors produced by the pathogen, are transferred into the host cells to manipulate the host cell machinery thereby suppressing plant defense. Therefore, studying the interface between the host and the pathogen at the early stages of infection is of great interest. An important drawback when studying the *Phytophthora*–host interaction in leaves is the lack of synchronization of the infection process. For this purpose, a new *in vitro* infection system was established, in which MsK8 tomato cell suspensions were challenged with zoospores of different *Phytophthora* species. Here we show that *P. infestans* infects MsK8 cells in a similar fashion as tomato leaf tissue. In contrast, other *Phytophthora* species that are not pathogenic on tomato could not penetrate the MsK8 cells. Expression analyses of *Phytophthora* effector and tomato defense genes and various histological assays were performed to monitor *Phytophthora*–MsK8 interactions in more detail. In addition, multi-omic datasets were generated (i.e., transcriptome, metabolome, proteome), and are currently integrated and analyzed in a systems biology approach to elucidate the essential processes during the *Phytophthora*–MsK8 interaction. The use of this novel infection system allows simplification and synchronization of the infection process, and is expected to provide a more detailed insight into *Phytophthora*–host interactions.

P62 CRISPR-Cas9 as a tool for the study of effector gene families in the plant pathogenic fungus *Ustilago maydis*

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All plant pathogenic microbes modulate the interaction with their respective host plants via secreted protein effectors. Bacterial plant pathogens deliver about 30–40 effectors via type III secretion systems to the cytosol of their host. In contrast, do fungal plant pathogens possess large effectomes of, usually, several hundred proteins? Some of these effector genes are members of multi gene families, a feature which greatly complicates their functional analysis. *Ustilago maydis* is the causative agent of corn smut disease. This basidiomycete fungus has become one of the fungal model organisms for studying biotrophic plant–pathogen interactions. The effectome of this fungus comprises 320 proteins. In our present work we have performed an in silico analysis of the *U. maydis* effector families. In addition, we have established CRISPR-Cas9 genome editing as a tool for reverse genetics in this organism. We present how this tool can be applied for the functional analysis of effector gene families.

P63 Conserved effectors in symbiotic AM fungi

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Studies of plant–microbe interactions prove a key role of effector proteins during host colonization. Pathogen effectors are released to alter host cell function and suppress the host immune system allowing successful infection. There are indications that symbiotic effectors may similarly influence the host defence and affect colonization. We hypothesize that effectors also play a crucial role in symbiosis between arbuscular mycorrhizal fungi (AMF) and plants. The conservation level of effector proteins between AMF species may indicate their fundamental function during symbiosis.
We predicted effector candidates and investigated their conservation in two closely related AMF species, *R. irregularis* and *R. clarus*. The putative effectomes were predicted based on the observation that known pathogenic effectors are secreted and fulfil at least one of the following criteria: are nuclear localized (NLS), are small and cysteine rich, contain internal repeats or show similarity to proteins expressed in haustoria. Based on the similarity level between both species we divided effector candidates into conserved (>90%) and non-conserved ones. Members of the NLS group are highly enriched among the conserved candidates and show the highest conservation level (62%) in comparison to the other groups. Therefore, plant nucleus localized candidates appear to be subject of evolutionary sequence conservation.

P64 Natural defense stimulation in wheat against *Mycosphaerella graminicola* using plant-growth-promoting rhizobacteria

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Septoria leaf blotch (SLB) caused by *Mycosphaerella graminicola* is one of the most devastating wheat diseases. In this work, we investigated the protective effect of the PGPR *Paenibacillus* sp. strain B2 and its lipo-polypeptide elicitor against SLB. The interaction between *Paenibacillus* B2 and *Curtobacterium plantarum* bacterium, found to be associated with almost all wheat seed cultivars, was also evaluated.
Results showed that *Paenibacillus* B2 and *C. plantarum* are endophytes and, only after co-inoculation of wheat pre-germinated seeds at sowing, significantly increased roots and aerial part fresh weights. Plant roots inoculation with 10^6 CFU of *Paenibacillus* B2 showed more than 70% of protection against SLB and paenimyxin showed >80% of local and systemic protection. The induced systemic resistance (ISR) as response to *Paenibacillus* B2 root inoculation is characterized by the over expression of *pr1, lox and peroxidase* genes. The local acquired resistances (SAR) as response to paenimyxin pre-treatment are characterized by the overexpression of *pal, chs, oxo, gst, aos, lox* and *β 1,3 glucanase,* genes and the systemic (SAR) by *chs*. The mechanisms potentially involved in plant protection are discussed.

P65 Pharmacological evaluation of submerged plant (*Typha latifolia* L.) of Pakistan

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Typha latifolia, a potent biodegradable plant, many of the pharmacological actions such as antimicrobial (antibacterial and antifungal), phytochemical constituents and antioxidant activities were evaluated. Results revealed antibacterial and antifungal well defined zone of inhibition. In this case *Typha latifolia* methanol root and fruit extract showed maximum value of zone of inhibition against *Xanthomonas axonopodis* and *Bordetella pertussis.* While, in the case of fungus *Penicillium italicum* root and fruit extract showed maximum value of zone of inhibition and Chloroform fruit extract showed maximum value of zone of inhibition against *Botrytis cinreae*. Overall the plant is very active for the production of secondary metabolites and positive result were recorded for alkaloids, terpenoids, flavonoids, tannins, phlobotannins and cardiac glycosides whilst, negative for others. Besides, the total antioxidant assay and DPPH assay was also performed in which all the plants parts extracts in different solvents showed different values accordance to the available standards as BHT and α-Tocopherol. Moreover, novel approach was also employed to evaluate the efficiency of these extraction methods viz., microwave assisted extraction and cold maceration to extract the phytochemicals i.e., phenolics from the *Typha latifolia* plant. Conclusively, some plant parts possess antimicrobial activity properties that can be used to cure infectious diseases.

P66 Interactions between the endophytic *Fusarium solani* isolate, FsK, and *Lotus japonicus,* or the mycorrhizal fungus *Gigaspora gigantea*

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Fusarium solani strain K (FsK) is an endophytic fungus, isolated from the roots of tomato plants grown on suppressive compost. The fungus has been recorded to confer resistance to the host plant, against both root and foliar pathogens. An attenuation of plant pathogenesis related genes – mediated by the endophyte – was reported to be independent of a pathogen's presence.

To understand the nature of endophytes, and their benefits to the plant, it is important to study their lifestyle in their various hosts. To this aspect, we studied the interaction of FsK with the model legume, *Lotus japonicus*, using Advanced Microscopy Techniques. A non-pathogenic GFP strain of FsK, carrying all the endophytic characteristics, was used. The endophyte colonizes the plant at the early stages of the interaction. The colonization pattern of the fungus is presented, as well as the cytological impact of the association on both partners.

In nature, endophytes interact, and/or overlap with other plant colonizers, for instance symbionts, pathogens, saprotrophs etc. To give an insight into the interplay of FsK with potential members of the root microbiome, the bipartite association of the former with an AMF strain (*G. gigantea*) is displayed, revealing differences to a pathogenic fungus–AMF interaction.

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P67 RNAse-like effectors in cereal powdery mildews: Evidence for the mode of action of BEC1054 on plant immunity

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Like many plant pathogens, the obligate biotrophic powdery mildews deploy a vast array of effector proteins that are produced at the infection interface between the host and microbe cells. In the powdery mildews, the largest super-family of effector genes encode RNAse-like proteins that are predicted to be secreted into the host cells. Silencing these effectors, such as BEC1054, results in reduced infectious development. Here, we show that expression of BEC1054 in *Nicotiana benthamiana* induced a significant increase in the susceptibility to *Peronospora tabacina*. The 3D structure of BEC 1054 is congruent with that of archetypal fungal RNAses from which these genes have evolved. However, BEC1054 does is not capable of degrading RNA whilst we found that it retains the ability to interact with and bind RNA. We found that transgenic BEC1054 in wheat plants abolishes the specific jasmonate-induced degradation of ribosomal RNA. Taken together, this data supports a model for the mode of action of RNAse-like effectors like BEC1054: they are produced by the obligate biotrophic powdery mildews to inhibit the jasmonate-induced RIP action which would otherwise lead to host cell death and death of the pathogen.

P68 Assessing NLR diversity in wild tomato species

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NLRs (Nod-like Receptors) are Nucleotide-binding domain and Leucine rich Repeats (NBLRR)-containing proteins that are important in plant resistance signalling. Many of the known pathogen Resistance (R)-genes in plants belong to the NLRs. NLRs occur in large numbers, clustered on the genome and show great variation between and within species. To date a number of studies has shown long-term evolutionary relationships of NLRs and have shown that different mechanisms might have been in place to accomplish such great NLR diversity as can be observed today. Whether all copies are functional and what these functions are remains an open question.
Evolution and population theory predict that also in short term interactions functional genes that play such an important role in the plant–pathogen interaction should be under certain selective pressure. Limited data suggests that in the inbreeding plant *Arabidopsis thaliana* known R-genes show signs of positive selection. However, no data exists for whole systems, complex ecological systems or outbreeding plant species. Here we assess NLR diversity in a complex, outbreeding, wild tomato species. Using Rgene Enrichment Sequencing (RENSeq) we sequence R-genes in wild tomato populations, from known geographical locations and assess short term evolutionary changes. We can link these data to infection assay to understand plant and pathogen population dynamics and understand the evolutionary pressures driving the birth and death of NLR genes. Moreover, finding NLR under selection will allow identification of the potentially new and important R-genes. It can also be a means of selecting candidate genes to study molecular biology of NB-LRR signaling and their roles in immunity. Our study can pave the way for further evolutionary and functional studies in NLR biology and can be expended to many model and crop species.

P69 *Cercospora zeina CTB* gene expression profiles suggests an early role for cercosporin in grey leaf spot disease development in maize

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The maize foliar pathogen, *Cercospora zeina* causes the agriculturally devastating disease, grey leaf spot (GLS). Members of the genus *Cercospora* cause disease in a variety of economically important crops worldwide and their success is often attributed to their ability to produce the phytotoxin, cercosporin. Dogma suggests that cercosporin acts during a late stage of infection, killing host cells via the production of ROS; however, this important component of the plant–microbe interface is currently not well understood. The aim of this study was to determine whether selected cercosporin toxin biosynthetic (*CTB*) genes were expressed *in planta*. The *CTB* gene cluster was identified in *C. zeina* by mining of the draft genome assembly and thereafter annotated. Subsequently, a *C. zeina*-maize infection trial was conducted using a susceptible maize cultivar. Infected leaf material was harvested at various time points for RNA isolation and RT-qPCR performed. All *CTB* genes studied were expressed throughout GLS development, suggesting that cercosporin is produced *in planta* despite a lack of *in vitro* cercosporin production and that the toxin plays an important role throughout GLS disease progression.

P70 Plant growth-promoting bacterial strain, SA187, influences hormonal pathways in *Arabidopsis*

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The plant rhizosphere consists of a variety of microorganisms that can potentially help plants to adapt to their particular environmental conditions. In order to isolate beneficial microbes that enhance the tolerance of plants to heat, drought or salt stress, the DARWIN21 project assembled a collection of more than 1000 endophyte bacterial stains from desert pioneer plants. Screening of the collection for enhancing salt tolerance of Arabidopsis plants revealed a number of beneficial endophytes. Greenhouse and field tests on wheat, barley and pearl millet provided proof that some of these endophytes can enhance the crop yield under extreme environmental conditions.
To better understand how beneficial endophytes enhance plant abiotic stress tolerance, we started a molecular genetic analysis with strain SA187. Genome sequencing revealed that SA187 is a novel alpha-proteobacterial species. Imaging showed that cells of GFP-labelled SA187 attach to *Arabidopsis* root surfaces, thereby establishing stable colonies. Infected seedlings exhibit shorter primary roots, a higher number of lateral roots and longer root hairs. In agreement with RNAseq analysis, fluorescent reporter lines revealed that SA187 may change plant hormone pathways. In conclusion, SA187-Arabidopsis interaction represents a good model system to elucidate mechanisms how endophytes enhance stress tolerance of plants.

P71 *PAD4*, *LSD1*, and *EDS1* regulate drought tolerance, biomass production, and cell wall properties of trees

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Tolerance increase towards environmental stresses would open opportunity for plant cultivation in these areas that were previously considered as ineligible, e.g. in areas with poor irrigation. We performed functional analysis of proteins encoded by *PHYTOALEXIN DEFICIENT 4*, *LESION SIMULATING DISEASE 1* and *ENHANCED DISEASE SUSCEPTIBILITY 1* genes to explain their role in drought tolerance and biomass production. *Arabidopsis* mutants *pad4-5*, *lsd1-1, eds1-1* and transgenic poplar lines *lsd1, eds1,* and *pad4* were examined in terms of morphological and physiological parameters. We proved that *Arabidopsis* PAD4, LSD1 and EDS1 play a role in survival under drought and regulate vegetative and generative growth. Improved water usage was also the outcome of LSD1 and PAD4-dependent regulation in poplar. Furthermore, biomass production and properties of poplar wood were orchestrated *via* a genetic system of *PAD4*/*LSD1/EDS1* which balanced the cell division and cell death processes. Our results demonstrate that PAD4/LSD1/EDS1 constitute a molecular hub, which integrates plant acclimation and developmental responses. The applicable goal of our research was to generate transgenic plants with regulatory mechanism that perceives stress signals to optimize growth and biomass production in semi-stress field conditions.
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P72 The putative host uptake motif in the Tin2 effector of *Ustilago maydis*

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The secreted Tin2 effector protein of the corn smut fungus *Ustilago maydis* is taken up by plant cells and induces anthocyanin biosynthesis to promote virulence. Presently, it is unclear how Tin2 is taken up. We have mapped the putative uptake motif in Tin2 and found that the biological activity of Tin2 is severely reduced when negatively charged residues downstream of the signal peptide are substituted by alanine. On the other hand, the substitution of positively charged residues to alanine did not affect the biological activity of Tin2. Protein stability of Tin2 carrying the substitution of negatively charged residues during plant colonization was similar to the wild type Tin2 protein. We are currently assessing whether the reduced biological activity is due to reduced uptake by using a biotinylation-based assay we have recently developed.

P73 Efficient silencing of *PEX6* in *Fusarium oxysporum* confers reduced sporulation and pathogenicity

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Peroxisomes are single-membrane-bound organelles that play a pivotal role in all eukaryotic cells in various developmental processes. We have identified *PEX6*, encoding peroxisomal biogenesis factor 6, in *Fusarium oxysporum* f.sp. *lycopersici*, a soil born phytopathogenic filamentous hemibiotrophic fungus that invades tomato roots and colonizes the xylem vessels, thereby causing complete wilting of infested tomato plants. *FoPEX6* was found to be up-regulated during early stages of growth and development of *F. oxysporum*. In this study, the effect of silencing of *FoPEX6* on sporulation and fungal pathogenicity was examined through RNA interference (RNAi). *FoPEX6* was isolated and a hairpin RNAi construct was prepared and then it was introduced into Fo4471 strain of *F. oxysporum* through glass-bead method. Transgenic status of fungal transformants was confirmed by molecular analysis. Silenced fungal transformants exhibited a significant reduction in sporulation as compared with wild-type strain. They also showed dramatic reduction in virulence on tomato, both in root infection and fruit tissue-invasion assays. These results suggest that peroxisomal biogenesis factor 6 has role in development, sporulation and pathogenicity in *F. oxysporum.*

P74 Cytokinins as virulence factors for the pathogenic fungus *Leptosphaeria maculans*?

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Hormones, small signal molecules, are a key component of plant defence against biotic and abiotic stresses. Different hormones form a complex network that enables coordination of defence with other plant processes, such as growth and development. Many microorganisms associated with plants evolved strategies how to disturb this hormonal balance. Some microorganisms inhibit hormonal biosynthesis, metabolism or signaling in plants due to effector molecules. Others can biosynthetise and secrete molecules identical with plant hormones. Oilseed rape (*Brassica napus*) is economically important crop. Among its most destructive diseases worldwide belongs stem canker ("blackleg") caused by *Leptosphaeria maculans*, a fungal hemi-biotrophic pathogen belonging to the Dothideomycetes. Quantification of hormones by LC-MS in the mycelium of *L. maculans* grown *in vitro* revealed a wide range of cytokinins produced by this fungus. The content and range of cytokinins differed between the fungus and the *B. napus* leaves and was further altered upon the infection with *L. maculans*. A possible role of cytokinins as a virulence factor motivated the study of cytokinin metabolism in *L. maculans*. Based on the gene orthology, the genes involved in cytokinin biosynthesis and metabolism were searched in *L. maculans* genome and functionally characterized by gene silencing.

P75 Multiple *Xanthomonas* type III effectors target the ubiquitin-proteasome system at different cellular localizations

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Xanthomonas campestris pv. *vesicatoria* (Xcv) translocates about 30 type-III effector proteins (T3E) into the host cell to cause disease. These T3Es localize to various cellular compartments to manipulate processes involving secretion, the ubiquitin-proteasome system (UPS) and gene expression. Evidence is emerging that manipulation of the UPS might be an effective and widespread virulence strategy of bacterial invaders to promote pathogenesis. In line with this, we could show that membrane-localized T3E XopJ, promotes virulence through the inhibition of the proteasome and a resultant suppression of salicylic acid (SA) – dependent defense. XopJ recruits proteasomal subunit RPT6 to punctuate structures at the plasma membrane being reminiscent of lipid rafts. At the plasma membrane, XopJ acts as a protease to degrade RPT6 triggering proteasome malfunction. Consequently, XopJ-mediated suppression of the proteasome impairs the proteasomal turnover of NPR1 leading to its accumulation. Preliminary analysis of the XopJ-induced ubiquitylome revealed candidates implicated in UPS, vesicle trafficking and calcium signalling processes. Characterization of other Xcv T3Es revealed effectors localized in the nucleus that interact with UPS components to stabilize transcription factors. Thus, Xcv evolved various T3Es that are present in different cellular compartments, but collapse on a single target process in their host cell.

P76 Nuclear pores enable robust perinuclear calcium signalling

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Nuclear Ca^{2+} signalling lies at the core of several of processes in plants – such as symbiosis. But despite recent experimental advances, the autonomy of nuclear calcium signaling remains an outstanding question in Plant Sciences.
The comparison between cytosolic and nuclear calcium oscillation patterns gives unclear results: whereas persistent gradients and significant delays have been reported in tobacco cells, simultaneous transients are observed during legume symbiosis. Although the observation of calcium transients in isolated nuclei of tobacco cells indicate that the nucleus has the minimal components to generate its own signals, similar components are found on both sides of the nuclear envelope, which is permeated by large pores connecting the nucleus with the cytosol.
Mathematical modeling can provide the necessary toolbox to investigate the concerted action of multiple signaling pathways. We adapt the fire-diffuse-fire model to clarify the autonomy of nuclear calcium signaling. We show how the existence of a calcium signaling machinery on both sides of the nuclear envelope can allow for distinct cytosolic and nuclear calcium signatures, and that pores are a necessary component for robust signaling over a wider range of conditions.

P77 Competition for sugars at the *Arabidopsis thaliana*/*Botrytis cinerea* interface

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The necrotrophic fungus *Botrytis cinerea*, the causing agent of the grey mold disease, is responsible for serious crop losses worldwide due to its large host spectrum. While signalling pathways leading to the PAMP-Triggered Immunity against *B. cinerea* are now well established, studies describing the role of sugars and their related transporters in plant defense are still scarce. It is postulated that sugar mobilization upon pathogen infection might participate in the plant immunity by providing carbon sources and energy. In *A. thaliana*, the active import of hexoses is known to be mediated by Sugar Transport Proteins (STPs) whereas hexose diffusion was recently imputed to the newly discovered SWEET proteins. We have developed a new system of interaction in which plant and fungus cells are physically separated by a semi-permeable membrane allowing molecular communication. This system permits the precise assessment of several parameters independantly for both plant and fungus cells. We studied the kinetics of sugar uptake upon *B. cinerea* infection and monitored changes in the sugar content as well as the sugar transporter gene expression. The results provided by this work provide new insights into the sugar competition taking place at the plant/pathogen interface.

P78 Can plants still benefit from AM fungi under carbon limiting conditions?

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It has previously been shown that the symbiosis between the AM fungus *Rhizophagus irregularis* and *Medicago truncatula* results in a beneficial situation for both partners. However, it is currently unknown if plants can actively reduce the carbon flow to the fungus, when a former symbiotic and beneficial situation turns into a less beneficial interaction due to carbon starvation.
To answer the question an experimental set-up was chosen where plants in a fully established symbiotic interaction were subsequently shaded, to induce a situation where carbon becomes a limiting factor.
Using gene expression analysis (sucrose transporter), enzyme activity assays (sugar converting enzymes) and protein accumulation profiles of elements of the plant carbon transport mechanisms are analyzed to investigate, if the plant is able to control the carbon flux to the fungus actively.
Furthermore we want to investigate if the fungus can get parasitic if the carbon availability is limited.
Preliminary data show, plants still benefit from the fungus when the photosynthetically fixed carbon is extremely reduced.

P79 Fungal immune-suppressive small RNAs targeting host plant immunity

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Fungal pathogens release diverse biomolecules at the plant–microbe cell interface to support infection, such as plant cell wall-degrading enzymes, toxins, and protein effectors that manipulate host physiology and immunity. Recent observations have added small RNAs to the list of effector molecules that enter plant cells and suppress host immunity. *B. cinerea* secretes small RNAs (*Bc*-sRNAs) into host plants that hijack the plant RNA interference (RNAi) pathway to suppress host immunity genes [1]. Expression of *Bc*-sRNAs in *Arabidopsis* confirmed silencing of host genes including two *Mitogen-activated protein kinases* (*MAPK*), *AtMAPK1* and *AtMPK2*, involved in anti-*Botrytis* defense. Silencing of host immunity genes relied on *Arabidopsis* Argonaute 1 (*At*AGO1). Co-immunoprecipitation of *At*AGO1 at sites of *Botrytis* infection revealed binding of immune-suppressive *Bc*-sRNAs. Moreover, the *atago1-27* mutant allele exhibited reduced susceptibility towards *Botrytis* infection, while *Bc*-sRNA host target genes showed de-repression.
This novel type of small RNA-based virulence strategy of a fungal pathogen illustrated that natural cross-kingdom RNAi exist in plant–fungal interaction [2,3]. Whether other plant pathogens evolved small RNA-based infection strategies, and what might be the underlying cellular events of RNA transmission from pathogen into host cells, will be discussed.

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P80 *In planta* manipulation of a plant´s microbiome in nature

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Endophytic bacteria are believed to be beneficial for plant productivity and resistance, but for most systems the microbial contribution to the plant as a holobiont remains to be evaluated in nature. We used Antimicrobial Peptides (AMPs) for the targeted manipulation of the microbiome in the wild tobacco (*Nicotiana attenuata*), and investigated consequences of endophyte depletion of this wild plant species. The constitutive expression of a knottin like peptide from the common ice plant resulted in selective *in planta* activity against the growth promoting bacterium *Bacillus pumilus* without affecting the plant pathogen *Pseudomonas syringae* pv*. tomato* DC 3000. Transgenic plants were compared to isogenic control plants regarding growth performance, herbivore resistance and their native root associated microbial communities in multiple field experiments. Surprisingly, high-throughput 16S rDNA sequencing analysis was not able to discriminate between the genotypes. The inoculation with single, native endophytic bacteria revealed a high degree of heterogeneity among phylogenetically closely related isolates, regarding their resistance to the expressed peptide. This indicates that a plant microbiome can tolerate antimicrobial effects on a sub-OTU level, due to a highly diverse composition of bacterial strains, a trait, which is readily missed by the short amplicon length commonly used in high-throughput sequencing analysis.

P81 Exploring factors that influence the composition of endophyte communities in *Leptospermum scoparium*

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Leptospermum scoparium J.R.Forst. et G.Forst. var. *scoparium* or mānuka is a New Zealand indigenous shrub and well known for essential oils and production of a unique honey that contains antimicrobial properties. The population structure, diversity and function of the endomicrobiome in mānuka is largely unexplored. Bacterial and fungal endophytes associated with different plant tissues and plant maturities from diverse locations were arrayed using denaturing gradient gel electrophoresis (DGGE). Results showed that plant tissue (root, stem, leaf) was a main factor influencing microbial richness and community structure in mānuka. Leaf tissue displayed the highest diversity in total bacteria compared to other tissues (LSD, $P<0.05$). In contrast, the recovery of culturable bacteria showed the opposite result. Leaves were least rich in total fungi and a*lphaproteobacteria* (LSD, $P<0.05$). Plant maturity influenced bacterial and fungal communities (PERMANOVA, $P<0.005$) but did not influence microbial richness (LSD, $P>0.05$). Plant location influenced *gammaproteobacterial* communities which was the most abundant group and culturable members showed several beneficial activities, such as, ability to solubilise phosphate compounds, production of siderophores and biocontrol activity against phytopathogens. In conclusion, plant tissue and maturity were the major factors influencing endophyte communities *in planta*. More detailed knowledge on the composition of the mānuka core endomicrobiome will be gained by next generation sequencing.

P82 The role of GTR importers in rhizosecretion of glucosinolates in *Arabidopsis*

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Plant-derived metabolites in the rhizosphere are essential for pathogenic and symbiotic interaction between plant and soil-borne organisms. Studies have to a large extent focused on the biological roles of these compounds, whereas the transport pathway from synthesis site to rhizosphere remains elusive. Glucosinolates (GLS) and their degradation products are crucifer-specific defence compounds. Towards our goal of understanding the transport pathway underlying root exudation of GLS and their degradation products, we first developed a sampling method enabling analysis simultaneously of GLS and their degradation products in root exudates of the model plant *Arabidopsis*. Subsequently, we implemented the method on wild type plants and the double mutant of two GLS importers, *gtr1 gtr2*. We found that stele-synthesized aliphatic GLS in *gtr1 gtr2* mutant decreased in the root exudate compared with wildtype, whereas 4MSB GLS and indole GLS levels were similar in both genotypes. The degradation products of all aliphatic GLS but not indole GLS were reduced in the exudates of *gtr1 gtr2* mutant. As a first step towards understanding the GLS root exudation process, we found that GTR-mediated retention of aliphatic GLS is prerequisite for exudation of stele-synthesized GLS and GLS degradation products to the rhizosphere.

P83 Role of symbiosis-regulated plant cell wall degrading enzymes from *Laccaria bicolor* in ectomycorrhiza development

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Plant-associated fungi have evolved a repertoire of enzymes acting on plant polysaccharides to colonize their hosts. The genomes of the ectomycorrhizal fungi sequenced so far have a reduced complements of plant cell wall degrading enzymes (PCWDEs) [1, 2, 3, 4]. Using comparative analyses of available genomics and transcriptomics data, we have identified a set of carbohydrate-active enzymes (CAZymes) that are released by the ectomycorrhizal basidiomycete *Laccaria bicolor* upon symbiosis development. The few retained genes coding for PCWDEs are acting on pectins (GH28, GH88 and CE8), hemicelluloses (GH30) and cellulose (GH5_5 with a CBM1 domain and LPMOs) and are upregulated in ECM root tips. They likely modify the plant cell walls during colonization of the host root apoplastic space. To characterize the enzyme activity and substrate(s) of the symbiosis-induced PCWDEs, we are producing the recombinant proteins for the unique glycosyl hydrolase 5 (GH5) with a carbohydrate-binding motif CBM1, an expansin-like protein and the polygalacturonase GH28. As of today, the GH5-CBM1, its catalytic motif, and the expansin-like protein have been produced in the yeast *Pichia pastoris*. The recombinant proteins are used for assaying the enzyme activity, determining the protein 3D structure and to elicit antibodies for further protein immunolocalization in ectomycorrhizal roots. This project will elucidate how symbiotic fungi modify plant cell walls to successfully establish within host tissues. In addition, it may generate new enzymatic tools for green chemistry.

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