DPG | Spectrum Phytomedizin

Matthias Hahn (Hrsg.)

55. Jahrestagung des DPG-Arbeitskreises Mykologie und 39. Jahrestagung des DPG-Arbeitskreises Wirt-Parasit-Beziehungen 2019

Zusammenfassungen der Arbeitskreisbeiträge

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Deutsche Phytomedizinische Gesellschaft e.V.

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2019

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21./22. März 2019

TU Kaiserslautern

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Arbeitskreise ‚Mykologie' und ‚Wirt-Parasit-Beziehungen' 2019

Die gemeinsame Tagung der Arbeitskreise ‚Mykologie' und ‚Wirt-Parasit-Beziehungen' fand am 21./22. März 2019 an der TU Kaiserslautern statt.

Die nächste Tagung ist für den 19./20. März 2020 an der TU München geplant.

Die Zusammenfassungen eines Teils der Beiträge werden - soweit von den Vortragenden eingereicht - im Folgenden wiedergegeben.

Leiter AK ‚Wirt-Parasit-Beziehungen': Matthias HAHN, Kaiserslautern
Leiterin AK ‚Mykologie': Anne-Katrin MAHLEIN, Göttingen

Bericht zum Jahrestreffen 2019 der Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“ der Deutschen Phytomedizinischen Gesellschaft

Das Jahrestreffen 2019 der Arbeitskreise „Wirt-Parasit-Beziehungen“ und „Mykologie“ der Deutschen Phytomedizinischen Gesellschaft e. V. fand am 21. und 22. März 2019 bei herrlichem Frühlingswetter an der Technischen Universität Kaiserslautern statt. Gastgeber war Professor Dr. Matthias Hahn, der das Treffen hervorragend organisierte und sich mit seinen Mitarbeiterinnen und Mitarbeitern sehr gastfreundlich um das leibliche Wohl der Teilnehmer kümmerte.

Das Jahrestreffen der beiden Arbeitskreise wurde in einer gemeinsamen Arbeitssitzung am ersten Tag, einer getrennten morgendlichen Sitzung am zweiten Tag und einer gemeinsamen Abschlusssitzung durchgeführt. Insgesamt waren ca. 100 Wissenschaftlerinnen und Wissenschaftler zum gemeinsamen Treffen nach Kaiserslautern gekommen. Unter ihnen war der wissenschaftliche Nachwuchs mit aktiven Beiträgen besonders stark vertreten. Insgesamt wurden 29 Vorträge gehalten. Darüber hinaus wurde in diesem Jahr die Möglichkeit Forschungsarbeiten in Form von Postern zu präsentieren und die Resultate mit Kollegen zu diskutieren, an beiden Tagen besonders eifrig genutzt (insgesamt 30 Poster der Arbeitskreise „Wirt-Parasit-Beziehungen“ und „Mykologie“). In der gemeinsamen Auftaktsitzung wurde außerdem der diesjährige Wissenschaftspreis der DPG vom Vorsitzenden der DPG, Dr. Gerd Stammler, an Prof. Dr. Ulrich Schaffrath (RTWT Aachen) überreicht.

Die in den Beiträgen vorgestellten Themen waren vielfältig. Sie umfassten beispielsweise Studien zu Veränderungen der Genexpression sowie Proteom- und Sekretomanalysen bei der Pathogenese von Pilzen, Oomyceten und Nematoden. Darüber hinaus behandelten sie die Identität und Wirkung von pilzlichen Effektoren und Pathogenitätsfaktoren, Wirt- und Nicht-Wirt-Resistenz von Pflanzen sowie die Induzierte Resistenz. Möglichkeiten von nicht-invasiven Sensoren zur Detektion von pilzlichen Schaderregern auf unterschiedlichen Skalenebenen wurden vorgestellt. Studien zu RNAi-Verfahren zur Schaderregerkontrolle und Beiträge zu Neuen Züchtungstechnologien ergänzten das wissenschaftliche Programm.

Das nächste gemeinsame Jahrestreffen der Arbeitskreise „Wirt-Parasit-Beziehungen“ und „Mykologie“ wird am 19. und 20. März 2020 an der Technischen Universität München stattfinden; Gastgeber wird Professor Dr. Ralph Hückelhoven sein.

Prof. Dr. Anne-Katrin Mahlein und Prof. Dr. Matthias Hahn

Deutsche Phytomedizinische Gesellschaft e.V.

Jahrestreffen der Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“ 2019

Programm

Donnerstag, 21.3.19 (TU Kaiserslautern, Geb. 57, Rotunde: Beide Arbeitskreise gemeinsam)

13:00 Uhr **BEGRÜSSUNG**

13:10 Uhr Pathogen small RNAs as host immune suppressors
Arne Weiberg (LMU München)

13:30 Uhr Bioinformatics tools for identification of candidate sRNA effectors in bidirectional cross-kingdom RNA communication between plants and plant microbes
Ena Secic, Karl-Heinz Kogel (Univ. Gießen)

13:50 Uhr Generation of recessive disease resistance by using CRISPR/Cas genome editing in oilseed rape (*Brassica napus*) genome
Michael Pröbsting, Dirk Schenke, Steffen Rietz, Daguang Cai (Univ. Kiel)

14:10 Uhr Identification of *Fusarium* infection, fungal DNA and mycotoxin contamination in wheat kernels and flour by hyperspectral imaging
Elias Alisaac, Jan Behmann, Petr Karlovski, Heinz-Wilhelm Dehne, Anne-Katrin Mahlein (Universität Bonn, Universität Göttingen, IFZ Göttingen)

14:30 Uhr Parasitic worms redirect host metabolism via NADPH oxidase-mediated ROS to promote infection
M. Shamim Hasan, D. Chopra, C. Matera, O. Chitambo S. Valerie-Mahlitz, S. Janakowski, M. Sobczak, A. Mithöfer, T. Kyndt, F. Grundler, S. Siddique (Univ. Bonn et al.)

14:50 Uhr *Colletotrichum* spp. from soybean cause disease on lupin but can also induce plant growth-promoting effects
Louisa Wirtz[1], Nelson Massola Júnior[2], Renata Linhares[2], Brigitte Ruge-Wehling[3], Ulrich Schaffrath[1], Marco Loehrer[1] ([1]RWTH Aachen, [2] São Paulo, [3]JKI Groß Lüsewitz)

15:10 Uhr **KAFFEEPAUSE & POSTER-PRÄSENTATIONEN**

16:10 Uhr The Link: Plant immune responses and their epigenetic regulation
Aline Koch (Univ. Gießen)

16:30 Uhr Mining and systematic analysis of the effector repertoire of *Ustilago hordei* during host colonization
Bilal Ökmen, Gunther Döhlemann (Univ. Köln)

16:50 Uhr Causes and mechanisms for alterations in the sensitivity of *Cercospora beticola* towards DMI fungicides
Maximilian Müllender, Gerd Stammler, Anne-Katrin Mahlein, Mark Varrelmann (IFZ Göttingen, BASF)

17:10 Uhr Deciphering the mode of action of the fungal germination inhibitor scopoletin
Verena Wanders, S. Kind, D. Spencer, G. Beckers, U. Conrath, C. Langenbach (RWTH Aachen)

17:30 Uhr Molecular characterization of the phytotoxic protein Hrp1 from *Botrytis cinerea*
David Scheuring, Tanja Jeblick, Thomas Leisen, Matthias Hahn (TU Kaiserslautern)

17:50 Uhr Mycoviruses in the rust fungus *Uromyces fabae*
Janina Seitz, Ralf T. Vögele, Tobias Link (Univ. Hohenheim)

18:10 Uhr Hinweise für das gemeinsame Abendessen

ab 19:00 Uhr GEMEINSAMES ABENDESSEN (Pizzeria Milano, Schoenstr. 15)

Deutsche Phytomedizinische Gesellschaft e.V.

Freitag, 22.3.19 (AK Wirt-Parasit-Beziehungen: Geb. 57, Rotunde)

8.30 Uhr AtGLP5, a germin-like protein of *Arabidopsis thaliana*, is a novel player in plant resistance mechanisms
Yan Zhao, Ronja Wonneberger, Wanzhi Ye, Zheng Zhou, Steffen Rietz, Daguang Cai (Univ. Kiel)

8:50 Uhr Role of the oligomerization of the B-lectin receptor kinase LORE in immune signaling
Sabine Eschrig, Stefanie Ranf (TU München)

9:10 Uhr Disclosing MPK interaction partners by XL-TAP-MS in *Arabidopsis thaliana*
Franz Leißing, Nicola Huck, Werner L., Huang L., Uwe Conrath, Gerold Beckers (RWTH Aachen)

9:30 Uhr Diversity and evolution of Resistance genes in wild tomato
Remco Stam (TU München)

9:50 Uhr Impact of small secreted maize proteins on pathogenicity during *Ustilago maydis* infection
Isabell-Christin Fiedler, Johannes Gössele, Karina van der Linde (Univ. Regensburg)

Freitag, 22.3.19 (AK Mykologie: Geb. 52, Hörsaal 52-206)

8.30 Uhr Comparison of different fungicide application criteria based on *Cercospora* leaf spot development and spore flight for *Cercospora* leaf spot control
Frederike Imbusch, Tobias Erven, Mark Varrelmann (IFZ Göttingen)

8:50 Uhr Sensitivity of grape powdery mildew (*Erysiphe necator*) towards demethylation inhibitors
Anna Huf, Gerd Stammler (BASF, Limburgerhof)

9:10 Uhr Hyperspectral imaging of sugar beet leaves in the UV range
Anna Brugger, Jan Behmann, Ulrike Steiner, Anne-Katrin Mahlein (Universität Göttingen, IZF Göttingen)

9:30 Uhr In-field detection of yellow and brown rust of wheat with hyperspectral sensors
David Bohnenkamp, Jan Behmann, Anne-Katrin Mahlein (Universität Bonn, IZF Göttingen)

9:50 Uhr *Trichoderma* spp. und Chitosan - Entwicklung einer Kombinationsstrategie zur Kupferreduktion im Weinbau
Verena Küpper[1], Ulrike Steiner[2], Bruno Mörschbacher[3], Andreas Kortekamp[1] ([1]DLZ, Neustadt a.d. Weinstraße; [2]Univ. Bonn; [3]Univ. Münster)

10:10 – 10.50 Uhr KAFFEEPAUSE / POSTER-PRÄSENTATION (Geb. 57)

Freitag, 22.3.19 (beide Arbeitskreise: Geb. 57, Rotunde)

10:50 Uhr *Arabidopsis thaliana* needs cell surface receptors for recognition of nonspecific elicitors of *Fusarium* spp.
Ralf Hückelhoven (TU München)

11:10 Uhr Tricky parasites: How nematodes take their vitamins from plants
Clarissa Hiltl, Florian Grundler (Univ. Bonn)

11:30 Uhr *Magnaporthe oryzae* HOG-signaling mutants as tools to explore mechanisms of fungicide resistance and host specificity
Stefan Bohnert[1,2], Florencia Casanova[2], Alex Wegner[2], Stefan Jacob[1,3], Ulrich Schaffrath[2], Eckhard Thines[1,3] ([1]IBWF Kaiserslautern; [2] RWTH Aachen; [3]Univ. Mainz)

11:50 Uhr Endophytic coming out: *Epichloë festucae* establishes an epiphyllous network on the surface of *Lolium perenne* leaves by development of an expressorium, an appressorium-like leaf exit structure
Yvonne Becker, Matthias Becker, Kimberly Green, Barry Scott (JKI Braunschweig)

12:10 Uhr Synthesis of α-1,3-glucan is required for cell wall function, hyphal polarity, differentiation of infection structures and full virulence of *Colletotrichum graminicola*
Maximilian Groß, Holger Deising (Univ. Halle-Wittenberg)

12:30 Uhr Optimising scopoletin biosynthesis for engineering disease resistance in crops
Alexander Beesley[1], Sebastian Beyer[1], Philipp F.W. Rohmann[1], Verena Wanders[1], Holger Schultheiss[2], Uwe Conrath[1], Caspar Langenbach[1] ([1]RWTH Aachen, [2]BASF Ludwigshafen)

12:43 Uhr Crop protection by secondary metabolism pathway engineering
David Spencer, Sebastian Beyer[1], Philipp F.W. Rohmann[1], Verena Wanders[1], Holger Schultheiss[2], Uwe Conrath[1], Caspar Langenbach[1] ([1]RWTH Aachen, [2]BASF Ludwigshafen)

12:55 Uhr Termin & Ort für Arbeitskreistreffen 2020, Verabschiedung

Zusammenfassungen der Beiträge

Pathogen small RNAs as host immune suppressors

Arne Weiberg
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Small non-coding RNAs (sRNA) are 20-30 nucleotides in length and mediate gene silencing via the RNA interference (RNAi) pathway, a conserved mechanism in eukaryotes. In plants, sRNAs are important regulators of innate immune response upon pathogen attack, while the role of sRNAs in microbial pathogens remains not well understood. Interestingly, mobile sRNAs can induce cell non-autonomous and systemic silencing in plants and animals. The broad-host fungal plant pathogen Botrytis cinerea delivers sRNA effectors into plants during infection, which hijack the host RNAi pathway to suppress plant innate immune response (1). This virulence mechanism is called cross-kingdom RNAi. Transport of extracellular RNAs was also reported from plants into pathogens and pests triggering host-induced gene silencing. We now discovered that the oomycete obligate biotrophic pathogen Hyaloperonospora arabidopsidis uses pathogen sRNAs that silence host mRNAs to establish infection in its plant host Arabidopsis thaliana. These discoveries imply that cross-kingdom RNA communication is common in plant-pathogen interactions. Our lab is interested in understanding the molecular principles of cross-kingdom RNAi and its implication on the evolutionary arms race between plants and pathogens. We will present new, unpublished data showing for the first time cross-kingdom RNAi in a plant-oomycete interaction. Further, we suggest that extracellular vesicles might be involved in pathogen sRNA transport in cross-kingdom RNAi. Weiberg et al. 2013 Science, 342: 118-123.

Bioinformatics tools for identification of candidate sRNA effectors in bidirectional cross-kingdom RNA communication between plants and plant microbes

Ena Secic, Karl-Heinz Kogel
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Small RNAs (sRNA) have been proposed as novel communication intermediaries between plants and microbes, utilizing the silencing machinery of RNA interference (RNAi) to modulate expression of genes involved in virulence or defense responses. Cross-kingdom RNA interference (ck-RNAi) is a bidirectional communication strategy known to exist in a few plant pathosystems (Weiberg et al., 2013; Zhang et al., 2016). Novel detection of this cross-talk strategy in plant – microbe interactions beyond these known examples is hampered by lack of consensus on a standardized workflow for detection and validation of sRNAs and their targets. Recently, we proposed a bioinformatics pipeline for detection of candidate sRNA effectors, prediction of their mRNA targets and the first in silico validation steps (Zanini et al., 2018). During this talk, the details and the application potential of this workflow on a broad plant-microbe interaction spectrum will be discussed. Zanini S, Šečić E, Jelonek L, Kogel KH (2018) A bioinformatics pipeline for the analysis and target prediction of RNA effectors in bidirectional communication during plant-microbe interactions. Front. Plant Sci. doi: 10.3389/fpls.2018.01212.

Generation of recessive disease resistance by using CRISPR/Cas genome editing in oilseed rape (*Brassica napus*) genome

Michael Pröbsting, Dirk Schenke, Steffen Rietz, Daguang Cai
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The genetic resource of resistance genes is limited in most crops and particular in oilseed-rape (*Brassica napus*). The breeding for resistant crops against new emerging pathogens remains a great challenge and requires the adaption of new technologies to improve this process. The phytopathogenic fungus *V. longisporum* is one of most important pathogens of oilseed rape and poses an increasing threat to oilseed-rape production, worldwide. The lack of genetic resources and the limitation of the application of fungicide ask for breeding for resistant oilseed rape. Recently, we identified a subset of genes and microRNAs in the oilseed rape genome, which are involved in the oilseed rape-*Verticillium* interactions. As the knockout of those genes enhanced plant resistance to the fungal infection in Arabidopsis, we assume that these genes are also required for oilseed rape susceptibility to *V. longisporum*, and therefore candidates for generation of recessive resistance against the fungus by using the CRISPR/Cas-based genome editing in oilseed rapeBrunner

. To this end, we have developed an efficient and codon optimized-expression cassette vector, which can be easily modified according to target genes as well as for the use of single/multiple sgRNAs. Meanwhile, we have generated several independent homozygous loss of function mutants for two candidate genes, respectively and are currently challenging those lines with the *V. longisporum* infection. Moreover, we have established a HDR (Homology Directed Repair)-based genome editing approach relying on the replication cycle of a disarmed gemini virus genome. This approach provides us a unique opportunity to interfere the plant-fungus interaction e.g. the miRNA-mRNA recognition to an interruption of the fungal infection process.

Parasitic worms redirect host metabolism via NADPH oxidase-mediated ROS to promote infection

M. Shamim Hasan[1], Divykriti Chopra[1], Christiane Matera[1], Oliver Chitambo[1], SinaValerie-Mahlitz[1], Slawomir Janakowski[2], Miroslaw Sobczak[2], Axel Mithöfer[3], Tina Kyndt[4], Florian M. W. Grundler[1], Shahid Siddique[1,5]
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Cyst nematodes, one of the economically most important groups of plant parasites, induce neoplastic syncytial nurse cells in roots of their host plants. Their invasion and feeding causes tissue damage in the host roots triggering an oxidative burst. In plants, ROS is mainly produced by plasma membrane-bound NADPH oxidases, named respiratory burst oxidase homolog (Rboh). Surprisingly, Arabidopsis mutants lacking ROS production by Rboh (rbohD/F) have been shown to be less susceptible to cyst nematode attack. A comprehensive microscopic, biochemical and molecular analysis has demonstrated that Rboh-dependent ROS are not required for Arabidopsis root invasion by cyst

nematodes; however, the absence of Rboh-mediated ROS impairs syncytium establishment and development. To understand the role of Rboh-mediated ROS in syncytium formation, we performed a genome-wide transcriptome analysis of Col-0 and rbohD/F upon nematode infection. Several genes involved in auxin transport, synthesis and/or homeostasis were down regulated in rbohD/F as compared to wild type. Notably, we identified WAT1, an auxin transporter, as one of the downstream targets of ROS. Hormone quantifications, metabolic profiling, genetic complementation and mutant analysis suggest that it regulates the pathways linking Rboh-mediated ROS to downstream responses. In summary, our work provides a first mechanistic understanding of the role of ROS in promoting infection of nematodes and other pathogens.

Colletotrichum spp. from soybean cause disease on lupin but can also induce plant growth-promoting effects

Louisa Wirtz[1], Nelson Sidnei Massola Júnior[2], Renata Rebellato Linhares[2], Brigitte Ruge-Wehling[3], Ulrich Schaffrath[1] , Marco Loehrer[1]

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The protein crop plants soybean and lupin attract increasing attention because of their use as fodder or green manure and for production of oil and protein for human consumption. While soybean production is recently gaining more importance in Germany and within the whole EU in frame of protein strategies, lupin production already is well established in Germany. The cultivation of lupins is impeded by the hemibiotrophic ascomycete *Colletotrichum lupini*, causing the recent outbreak of anthracnose disease. Soybean is also a host for a variety of *Colletotrichum* species. Because these pathogens are reported to have a broad host-range, cross-virulence on lupin may occur, especially taking into consideration the increasing vicinity of soybean and lupine cultivation in Germany. To address this question, we systematically investigated the interaction of (novel) *Colletotrichum* species isolated from soybean in Brazil on actual German soybean and lupin plant cultivars. Conversely, we tested the interaction of a German field isolate of *C. lupini* with soybean. Under greenhouse conditions *Colletotrichum* species from soybean and lupin were able to infect the respective other host plant with varying degrees of virulence. In addition, we observed distinct plant growth-promoting effects for some host-pathogen combinations which might open the route to novel approaches in lupine and soybean production.

The Link: Plant immune responses and their epigenetic regulation

Aline Koch

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Microrchidia (MORC) proteins comprise a family of proteins that are involved in gene silencing, transposable element repression, and multiple layers of immunity (Koch et al. 2017). Plant MORCs were discovered through a genetic screen for Arabidopsis mutants compromised for resistance to the turnip crinkle virus (Kang et al. 2008). MORCs also participate in pathogen-induced chromatin remodeling and epigenetic gene regulation. Animal MORCs exhibit many parallels with their plant counterparts, as they have been implicated in disease development and gene silencing (Koch et al. 2017). The discovery that AtMORC1 binds a wide variety of R proteins and the PRR (pattern recognition

receptor) FLS2, and that the MORC - R protein interaction is disrupted by R protein activation, gives a first hint on how ETI and PTI are influenced by MORC proteins (Langen et al. 2014). Thus, together with the finding that AtMORC1 shuttles from the cytoplasm to the nucleus after flg22 treatment (Kang et al. 2012), open the question whether MORC proteins represent the “linker” between pathogen recognition and defense gene regulation via their function in RdDM. Although the link between MORCs' dual role in immunity and TGS is currently unknown, the discovery that bacterial infection alters AtMORC1 binding at genomic regions preferentially associated with TEs provides an important clue (Bordiya et al. 2016). Here, we provide a mechanistic link between MORCs' role(s) as effectors/modulators of immune responses and epigenetic processes in plants that involves SA-mediated nucleocytoplasmic trafficking of R proteins, thus controlling transgenerational systemic acquired resistance.

Mining and systematic analysis of the effector repertoire of *Ustilago hordei* during host colonization

Bilal Ökmen, Gunther Döhlemann
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The success of plant pathogenic fungi relies on their arsenal of virulence factors that are expressed and delivered into the host tissue during colonization. The biotrophic fungal pathogen *Ustilago hordei* causes covered smut disease on both barley and oat. In this study, we combined cytological, genomics and molecular biological methods to achieve a better understanding of the molecular interactions in the *U. hordei*-barley pathosystem. Microscopic analysis revealed that *U. hordei* densely colonizes barley leaves upon penetration, in particular the vascular system, and forms haustorium-like structures. Transcriptome analysis of *U. hordei* at different stages of host infection revealed differential expression for 273 effector gene candidates. Furthermore, *U. hordei* transcriptionally activates core-effector genes that may suppress even non-host early defense responses. Based on expression profiles and novelty of sequences, knock-out studies of 14 effector candidates were performed in *U. hordei*, which resulted in identification of four virulence factors (Uvi1 to 4) required for host colonization. Yeast-two-hybrid screening performed for the Uvi1-4 identified potential barley targets for two of the effectors. Moreover, we have also characterized a secreted *U. hordei* ribonuclease protein that induces plant cell death in tobacco leaf. Together, this study provides a first systematic analysis of the effector repertoire of *U. hordei* and identified four effectors (Uvi1 to 4) as virulence factors for the infection of barley.

Deciphering the mode of action of the fungal germination inhibitor scopoletin

Verena Wanders*, Sabine Kind, David Spencer, Gerold Beckers, Uwe Conrath & Caspar Langenbach
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Asian soybean rust (SBR) is caused by *Phakopsora pachyrhizi*, an obligate biotrophic fungus that causes severe economic losses in all major areas of soybean production. Currently *P. pachyrhizi* is almost exclusively controlled by synthetic fungicides to which the fungus quickly develops insensitivity. Inhibiting key cellular signaling mechanisms in the fungus could be an alternative means of Asian soybean rust control. However, to date little is known about cellular signaling mechanisms in *P. pachyrhizi* and other rust fungi. We first characterized activation of different cellular processes

during *P. pachyrhizi* pre-infection structure formation, including nuclei migration, cytoskeleton dynamics, accumulation of reactive oxygen species (ROS) and mitogen-activated protein kinase (MAPK) phosphorylation. We then compared the influence of different inhibitors and the plant defense-associated spore germination inhibitor scopoletin on *P. pachyrhizi* physiology during pre-infection structure formation to illuminate the coumarin' s mode of action in germination inhibition. Due to its likely distinct mode of action from currently applied fungicides, scopoletin may be useful to complement current SBR management strategies.

Molecular characterization of the phytotoxic protein Hrp1 from *Botrytis cinerea*

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Necrotrophic fungi secrete an array of cell wall-degrading enzymes, proteases and toxins. Loss-of-function studies with proteins of the VELVET family in *B. cinerea* revealed an impaired acidification ability and a strongly reduced virulence. Quantitative transcriptome and secretome studies showed differential gene expression of secreted proteins in the VELVET mutants, and a predominantly transcriptional regulation of protein secretion. Downregulation of major cell wall-degrading enzymes and proteases support a crucial role of these protein classes for the infection process.

Based on these data we started a screen to find novel virulence determinants, focusing on secreted phytotoxic or defence-inducing proteins. Overall, we could identify more than 200 secreted *B. cinerea* proteins, including several already known toxic proteins. We narrowed down the number of potential candidates by using native 2D chromatography and found >25 toxic protein fractions which were further analyzed by MS-MS. Individual candidates were tested for toxicity, using Agrobacterium-mediated plant transformation. After identification of three yet undescribed phytotoxic proteins, we started to characterize the most promising one, the "hypersensitive response-inducing protein 1" (Hrp1). Hrp1 has no enzymatic activity, but seems to function as a PAMP-like signal which triggers simultaneously plant defence and apoptotic cell death.
Currently, *B. cinerea* gene knockout mutants for Hrp1 and other phytotoxic proteins are being generated and tested for their virulence, in order to decipher the functional composition of the entire phytotoxic secretome of *B. cinerea*.

Mycoviruses in the rust fungus *Uromyces fabae*

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Uromyces fabae is an obligate biotrophic rust fungus with an autoecious and macrocyclic life cycle. It is the causal agent of faba bean rust and a threat to important crop species like broad bean (*Vicia faba*), pea, and lentil. Mycoviruses are defined viruses that infect and replicate in fungi. They are common in all major taxonomic groups of true fungi as well as in plant pathogenic oomycetes which hints at millions of species. The study of mycoviruses has been going on for more than 50 years but until recently only roughly 100 mycoviruses were identified. With Next Generation Sequencing, sequence information for more species is elucidated. The roles of mycoviruses are largely unknown. To study the genomes of mycoviruses in *U. fabae*, dsRNA isolation was performed using the protocol of Morris and

Dodds (1979), which uses specific binding of dsRNA to cellulose in the presence of 16.5% ethanol. Electrophoresis showed seven distinct bands representing putative virus dsRNA. dsRNA was used to create a small scale cDNA library using the pJet1.2 cloning vector followed by Sanger sequencing. Additional sequence information was gained through RT-PCR. Finally, Illumina sequencing was performed. At this time, we have found at least 20 virus-like contigs with lengths between 1,113 bp and 10,755 bp, which are mostly characterized as totiviruses and show homology to other mycoviruses like *Puccinia striiformis* totivirus and Red clover powdery mildew-associated totivirus. The next step will be the performance of RACE-PCR to gain the missing sequence information at the contig ends. Also there are data indicating that we still do not have sequence information on all viruses present in *U. fabae*.

AtGLP5, a germin-like protein of *Arabidopsis thaliana*, is a novel player in plant resistance mechanisms

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The disease *Sclerotinia* stem rot, caused by *Sclerotinia sclerotiorum*, is one of the most important diseases of oilseed rape. Germin-like proteins are involved in plant resistance to the fungal infection in oilseed rape. The A. thaliana gene AtGLP5 is an ortholog of BnGLP3 of oilseed rape. While the overexpression of AtGLP5 in Arabidopsis resulted in plant resistance, the knockout of Atglp5 in Arabidopsis increased plant susceptibility to S. sclerotiorum, demonstrating the role of AtGLP5 in conferring plant resistance to the *S. sclerotiorum*. Recent transcript profiling and promoter-GUS assays revealed that the AtGLP5 is constitutively active in Arabidopsis roots, not in leaves. But, in leaves, the expression of AtGLP5 was activated by diverse a/biotic stress factors e.g. by *S. sclerotiorum* and *Pseudomonas syringae* infections as well as by wounding and salicylic acid treatments. Analysis of a series of 5' deletions of the promoter identified a 240bp core-promoter region that control major activities of the AtGLP5-promoter. Here, we demonstrate that AtGLP5 is a H2O2 generating superoxide-dismutase and transcriptionally activated/regulated by multiple signaling pathways, and for instance its expression in leaves was specifically activated in a Flg22/FLS2-depedent manner. Following this, we conclude that AtGLP5 is a novel player in plant innate immune responses. A possible functional model is discussed.

Disclosing MPK interaction partners by XL-TAP-MS in *Arabidopsis thaliana*

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Mitogen-activated protein kinase (MPK) cascades are essential for signal transduction by converting and amplifying environmental stimuli into diverse intracellular responses. MPK cascades are regulated through reversible phosphorylation of its components MEKKs, MEKs, and MPKs. Recently, we identified a high number of MPK substrates by combining tandem metal-oxide affinity chromatography (tandemMOAC) with stable 15N isotope labeling of Arabidopsis seedlings for accurate quantification of phosphopeptides by mass spectrometry. However, tandemMOAC does not provide protein-protein

interaction data. Thus, the type of interaction (direct or indirect) of the MEK, MPKs and their downstream substrates remains undefined. Here, we present a MS-based quantitative analysis of in vivo cross-linked and tandem affinity-purified protein complexes as a novel technique for identifying protein interaction partners. The XL-MS technique allowed us to disclose the identity of stable, weak, and transient interaction partners of the MKK2/MPK4 module. We could verify these interactions using split luciferase complementation assays. Additionally, by using in vitro kinase assays, we identified some of these interaction partners as substrates of MPK4. Taken together, we show, that this technique is a powerful method for the identification of in vivo protein interaction partners.

Diversity and evolution of Resistance genes in wild tomato

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While much work focuses on the identification of new pathogen Resistance (R) genes and resistance signalling pathways, less is known the diversity of defence processes within one plant species in their ecological settings. We study this diversity and the evolution of defence processes in the wild tomato species *Solanum chilense*. The species originated in a mesic environment and independently colonised a coastal and an arid highland habitat. We have shown that different populations of the species have differences in resistance properties against a range of important phytopathogens, including *Phytophthora*, *Alternaria* and *Fusarium* spp. To study the genetics underlying these differences. We generated a high-quality de novo reference genome of S. chilense and use targeted re-sequencing to extract the species' R genes and obtain polymorphism data over 14 populations. We estimate the demographic history of the species and identify those R genes under novel selection pressures in new habitats. We find that R genes causing adaptation to the main habitats belong to different functional clades and are more central in the resistance signaling network. R genes involved in adaptation to local environments and local pathogen strains belong to various functional clades that are less connected in the network. These findings highlight the complexity of R gene evolution within a single species and have implications for the search of durable R genes, to be used in plant breeding programmes.

Impact of small secreted maize proteins on pathogenicity during *Ustilago maydis* infection

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Maize is the most important cereal crop, and understanding the molecular processes orchestrating its reproduction and resistance is of huge agricultural relevance. In this regard, small secreted proteins (SSPs) from plants are promising subjects of study since they are emerging as potential key players in a variety of plant biological processes, e.g. development, response to abiotic stress, mediating pathogen recognition, and preventing invasion of the host. The biotrophic pathogen *Ustilago maydis* infects all aerial tissues of maize. Evidently, a correct organ-developmental programme is a prerequisite for infection. In order to identify novel SSPs, existing proteomic data sets of maize male flowers were filtered for candidates. For rapid screening of candidate proteins, the novel Trojan Horse (TH) approach was applied which utilizes *U. maydis´*s secretory capabilities. Using this technique, we identified new SSPs that confer enhanced resistance to *U. maydis*. Latest results from the screen and further in-depth candidate characterization will be shown.

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Sensitivity of grape powdery mildew (*Erysiphe necator*) towards demethylation inhibitors

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Grape powdery mildew (caused by *Erysiphe necator*) is one of the most important diseases in grapes worldwide. It is controlled by various fungicides including demethylation-inhibitors (DMIs). In this study we analysed the sensitivity of European isolates of *E. necator* to two DMIs. It turned out that two biotypes of *E. necator* were isolated, which could be identified by differences in their ITS-DNA sequence and their CYP51 haplotypes. Biotype A was rarely found and was more sensitive than biotype B. Within biotype B, two groups with different sensitivities could be distinguished. The group with lower sensitivity showed the mutation Y136F in the CYP51. Homologous mutations are known to cause lower DMI sensitivities in various fungal species, such as *Zymoseptoria tritici*, *Blumeria graminis* or *Phakopsora pachyrhizi*. Another interesting finding was that a variation in the gene copy number of the CYP51 gene could be detected in several isolates. It can be assumed that the gene amplification leads to an overall higher transcription rate and consequently to increased concentrations of the CYP51 enzyme in the cell, which might contribute to a higher DMI adaptation. However, as known for other pathogens, the target site mutation seems to have stronger effects on sensitivity reduction than target site overexpression.

A combination of UAV based hyperspectral imaging and modern data analysis could provide non-invasive disease detection for precision farming

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Multiple studies showed the potential of hyperspectral imaging for plant disease detection. Nevertheless, practical application of this technology in agriculture has not been established, so far. Hyperspectral measurements for early disease detection have been mostly performed on leaf scale, or in close distance to the canopy. These distances are impractical when entire fields have to be assessed.
This study presents a drone-based approach for hyperspectral imaging as method of disease detection and quantification, allowing rapid measurement of large areas in the field. In order to achieve this, hyperspectral measurements of multiple plant/pathogen interactions are performed and correlated with molecular analyses. The relevant information for pathogen detection of the resulting datasets can then be highlighted through the use of modern data analysis methods – in supervised and unsupervised approaches.
The goal of the study is to fly over entire fields with the drone-based hyperspectral measurement setup and automatically create disease maps, which can be used to precisely initiate plant protection measurements. This project has the potential to increase the efficiency in precision farming.

Trichoderma spp. und Chitosan - Entwicklung einer Kombinationsstrategie zur Kupferreduktion im Weinbau

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An effective and environmental friendly plant protection strategy is an important contribution to sustainable viticulture. The synergetic combination of biological and biochemical biostimulants, namely *Trichoderma* and chitosan, emerges as a new approach for copper reduction in organic crop management. The application of these biostimulants promotes plant growth and development. Additionally, it strengthens tolerance to abiotic and biotic stresses, such as Downy mildew infections. Both *Trichoderma* and chitosan are also able to act as bio-control agents inducing plant disease resistance. This might enable a significant copper reduction in viticulture. First investigations have shown an inhibition in development of *P. viticola* by the application of chitosan oligomers under laboratory conditions. Furthermore, field experiments showed a reduced infestation. To combine *Trichoderma*, chitosan and copper fungicides simultaneously, the compatibility of all used agents has to be guaranteed. For this purpose, copper tolerance in *Trichoderma* can be increased, but not to a level of field doses that are required for effective plant protection. On the other hand, chitosan is able to decrease the effective copper dosage, and *Trichoderma* spp. are known to release chitosanolytic enzymes so that a combination with antimicrobial chitosans is possible. First analysis identified six copper tolerant *Trichoderma* isolates out of 148 tested ones. They showed high mycelial growth and spore germination rates during cultivation with various copper concentrations. Additionally, a common copper fungicide was selected for the combination with *Trichoderma*.

Tricky parasites: How nematodes take their vitamins from plants

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Heterodera schachtii is a plant-parasitic nematode with an economically important impact on sugar beet production. The second-stage juveniles invade the root of their host and move intracellularly toward the vascular cylinder, where they induce the formation of a plant derived, hypertrophic, and hypermetabolic syncytium and become sedentary. The syncytium serves as the sole nutritional source for the developing juveniles. Due to this dependency, it is crucial for *H. schachtii* to successfully initiate and maintain the syncytium in order to complete its lifecycle and produce progeny. Vitamin B5 (VB5), the precursor to co enzyme A, is an essential nutrient for all animals. Plants, in comparison, can synthesize VB5 de-novo in a three-step pathway. The first committed step is performed by AtPANB1. Transcriptome data has revealed that AtPANB1 is significantly up-regulated in the syncytium, induced by H. schachtii in *Arabidopsis thaliana* roots. The essential role of this enzyme for cyst nematode infection was further confirmed, as AtPANB1 knock-out mutants were less susceptible to infection by *H. schachtii*. The last enzymatic step is performed by AtPANC, which is not differentially expressed in the syncytium, and the loss-of-function mutation had no effect on the parasitism of *H. schachtii*. Notably, our work has identified a nematode PANC gene (HsPANC), showing that nematodes are able to perform the last step of the VB5 biosynthesis using HsPANC. We assume that this compartmentalization between nematodes and syncytia circumvents feedback/feed-forward inhibitions to support continuous supply of VB5 to nematodes.

Endophytic coming out: *Epichloë festucae* establishes an epiphyllous network on the surface of *Lolium perenne* leaves by development of an expressorium, an appressorium-like leaf exit structure

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The biotrophic fungus *E. festucae* systemically colonizes the intercellular spaces of the aerial tissues of *Festuca* and *Lolium* grasses, including leaf primordia, sheath and blade tissue. Besides forming an endophytic hyphal network, *E. festucae* also grows as an epiphyte but the mechanism whereby it establishes a network on the surface of the leaf is not known. Using a combination of confocal laser scanning (CLS)-, scanning electron- and transmission electron- microscopy we have identified a novel structure, which we have named an expressorium to distinguish it from the appressorium used by plant pathogens to enter plants, that allows endophytic hyphae to exit to the leaf surface. The expressorium is a swollen hyphal compartment, often delimited by two septa, that develops just below the cuticle after the hyphae have passed through the epidermis. CLSM analysis of aniline blue/WGA-AF488 co-stained samples revealed a major remodelling of the fungal cell wall following exit from the leaf. Only the septa of endophytic hyphae fluoresce with WGA-AF488 whereas the entire cell wall of epiphytic hyphae fluoresce, suggesting cell wall chitin is either absent or masked in the former but not the latter; results consistent with the need to avoid a host defence response.

***Magnaporthe oryzae* HOG-signaling mutants as tools to explore mechanisms of fungicide resistance and host specificity**

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HOG (High Osmolarity Glycerol)-signaling is essential for osmoregulation in fungi and interferes with resistance to the fungicide fludioxonil. Thus, *Magnaporthe oryzae* (Mo) loss-of-function mutants of HOG-signaling components are osmosensitive and fludioxonil-resistant. We evidenced that homolog overexpression of a gene encoding the phosphatase MoPtp2 displays a new mechanism of fungicide resistance due to dephosphorylation of the HOG-signaling MAPK (Mitogen Activated Protein Kinase) MoHog1. Likewise, genetic inactivation of the phosphatase encoding gene MoPTP2 led to increased fludioxonil sensitivity in the mutant strain compared to the wildtype strain, thus expanding our knowledge of fludioxonil action. Investigation of another novel and potentially MoHog1 interacting protein, the transcription factor (TF) MoHot1, did not result in changes in osmostress- or fungicide-susceptibility of mutant strains. However, inactivation of the TF encoding gene MoHOT1 led to increased virulence on barley but not on rice in comparison to the wildtype strain. By contrast, overexpression of the MAPK encoding gene MoHOG1 led to drastically reduced virulence on barley but not on rice. The influence of altered HOG-signaling in the rice blast fungus has not been linked to host specificity so far. Delineating the reasons for these host plant specific virulence shifts will expand our understanding of plant-fungal interactions.

Synthesis of α-1,3-glucan is required for cell wall function, hyphal polarity, differentiation of infection structures and full virulence of *Colletotrichum graminicola*

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The cell wall of filamentous fungi is composed of structural elements embedded in amorphous polymers, the most prominent of which is α-1,3-glucan, comprising up to 40% of the whole carbohydrate fraction of the cell wall. Despite the enormous importance of a rigid and dynamically adapting cell wall, the role of α-1,3-glucan in vegetative and pathogenic development of plant pathogenic fungi is poorly understood. Our goal was to elucidate the roles of α-1,3-glucan in the maize anthracnose fungus *Colletotrichum graminicola* by functionally characterizing the three genes encoding α-1,3-glucan synthases, i.e. AGS1, AGS2, and AGS3. Our results indicate an important role of these genes in pathogenic and vegetative development, as the ags mutants show severe growth retardation and strongly reduced penetration rates on maize leaves. We hypothesize that these effects are caused by increased pore sizes in the cell wall of ags mutants due to the lack of α-1,3-glucan as a filling material which allows a higher mobility of cell wall-modifying enzymes.

Optimising scopoletin biosynthesis for engineering disease resistance in crops

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The coumarin scopoletin has multiple beneficial properties that promote its use in agriculture and as a nutraceutical. In plants, scopoletin contributes to the response to biotic and abiotic stress. However, to our knowledge successful application of the coumarin in plant protection has not been demonstrated so far. Here, we show that spray application of scopoletin to soybean leaves provided protection from Asian soybean rust by interference with the formation of *P. pachyrhizi*' s pre-infection structures. Consistent with its function in scopoletin biosynthesis, constitutive expression of Arabidopsis Feruloyl-CoA 6'-hydroxylase 1 (AtF6'H1) enabled production of scopoletin and its glycoside scopolin in transgenic Arabidopsis, tobacco and soybean plants and in tobacco BY-2 cell cultures. However, precursor feeding to AtF6'H1-overexpressing suspension cells increased scopoletin production, strongly suggesting a bottleneck upstream of F6'H1 in the phenylpropanoid pathway. To overcome AtF6'H1 substrate limitation in transgenic plants we co-expressed a transcription factor that globally triggered expression of secondary metabolism-associated genes and drastically increased accumulation of scopoletin and scopolin in transgenic plants. Currently, we are assessing the applicability of different promoter/transporter combinations for regulating temporal and spatial accumulation of scopoletin and avoiding detrimental effects of its hyperaccumulation in transgenic plants. Crops engineered this way will be tested for their tolerance to biotic and abiotic stress.

Crop protection by secondary metabolism pathway engineering

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Engineering crops for the enhanced accumulation of antimicrobial secondary metabolites is a promising means for sustainable disease management. Here, we show that stress-induced enzymes can be used to optimise the production of coumarins *in planta*. As a first step, we heterologously expressed an O-methyltransferase from sunflower (*Helianthus annuus*) in Escherichia coli. Substrate feeding assays revealed its activity towards different hydroxylated coumarins leading to biosynthesis of the antifungal methoxycoumarins scopoletin, isoscopoletin, and scoparone. Transient expression of the O-methyltransferase in *Nicotiana benthamiana* led to the accumulation of high amounts of the dimethoxylated scoparone. The latter coumarin was absent from non-transformed plants. Our results indicate that fine-tuning the secondary metabolism of crops by exploiting biosynthetic enzymes from different origins in the plant kingdom is a promising strategy for sustainable disease management.

PDF2.2 is an antifungal protein and involved in plant-*Verticillium* interactions

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Plant defensins proved to be functional in plant resistance against phytopathogenic fungi. In oilseed rape and Arabidopsis plants, we had observed that knockout of a candidate gene for susceptibility (CF-1) resulted in plant resistance to the *Verticillium longisporum* infection, accompanied with a strongly enhanced expression of PDF2.2. To identify the antifungal activity of this gene, we generated PDF2.2-overexpressing and knockdown Arabidopsis lines and challenged these with the fungal infection. As a result, the knockdown plants showed highly susceptible with much more pronounced fungal colonization and symptom development. By contrast, the overexpression lines were becoming resistant and showed impaired development of symptoms and fungal colonization as well. Transcript- und promoter-GUS histochemical staining assays revealed that the PDF2.2 expression levels were drastically changed during the infection process, firstly suppressed starting from hyphal penetration till 6dpi and then gradually elevated in the infected roots. These results support for our hypothesis that PDF2.2 is an antifungal protein and involved in plant resistance response to the *V. longisporum* infection and the suppression of PDF2.2 expression at the early infection stage might belong to the virulence strategy of *V. longisporum* to initiate/establish a compatible plant-fungus interaction.

Morphological and molecular characterization of *Diaporthe*/*Phomopsis* species associated with soybean seed decay

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The genus *Phomopsis* (teleomorph *Diaporthe*) comprises phytopathologically relevant fungi which cause diseases on a wide range of economically important crops. This group of pathogens has been reported to be involved in several soybean diseases, including Phomopsis Seed Decay (PSD) (*P. longicolla*), Stem Blight (*D. phaseolorum* var. *sojae*), and Stem Canker (*D. phaseolorum* var. *caulivora*, and *D. phaseolorum* var. *meridionalis*), resulting in significant yield and quality losses. Accurate species identification of the *Diaporthe*/*Phomopsis* Complex (DPC) is critical in understanding disease epidemiology and for developing effective control measures. In this study, we focused on morphological (color and shape of colonies, existence of alpha, or beta conidia, or both, and their characteristics, production of perithecia, and size of conidia) and molecular analyses of species from DPC-damaged European soybean seeds obtained from several locations throughout Austria, France, and Germany. It has been documented that MAT primers are useful in mating-type diagnosis in a wide range of *Diaporthe* and *Phomopsis* species. In addition, DPC isolates were therefore classified according to their mating-type loci using Primers MAT1-1-1FW/RV and MAT1-2-1FW/RV. Surface sterilized soybean seeds were placed on acidified potato dextrose agar and incubated for 30 d at 24°C. Putative DPC isolates were purified using the single spore method. Genomic DNA was extracted from mycelium of each single-spore isolate. Thirty-two strains of *Diaporthe* and *Phomopsis* were isolated and phylogenetic relationships were determined using the translation elongation factor 1-alpha (TEF1), beta-tubulin (TUB) and nuclear ribosomal DNA internal transcribed spacers (ITS) sequences. By combining morphological and molecular data, four species namely *Diaporthe longicolla*, *Diaporthe caulivora*, *Diaporthe novem* and *Diaporthe eres* could be distinguished on soybean seeds. Results from our mating-type experiments revealed that MAT primers used in this study allowed mating-type diagnosis of 29 isolates.

Nonhost resistance protein OsJAC1 and its domain orthologues confer resistance in mono- but not in dicotyledonous plants

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Plant pathogens are a constant threat for crop cultivation. The situation might become worse in the future because climate change and other anthropogenic factors favor the invasion of novel pathogen species. Nonhost resistance which protect plants against all non-adapted pathogens can be a valuable source for identifying novel resistance factors. In this context, OsJAC1, a protein from rice was identified to provide broad-spectrum disease resistance in barely, wheat and rice when overexpressed. It could be speculated that this might also help against invasive species. OsJAC1 is a fusion protein composed of a jacalin-related lectin (JRL)- and a dirigent (DIR)-domain only found in monocotyledonous plants. Over-expression of OsJAC1-GFP in barley but not in Arabidopsis led to enhanced resistance against adapted powdery mildew fungi. In Arabidopsis, genes were identified which encode for proteins with a single JRL- or DIR- domain. Transient co-expression of particular pairs of these genes also led to enhanced resistance against powdery mildew in barley. Interestingly, testing for interaction between these Arabidopsis JRL- and DIR proteins via bimolecular fluorescence complementation only led to positive results in barley but not in *Nicotiana benthamiana*. These findings strongly suggest that the resistance pathway involving JRL- and DIR- proteins only evolved in monocotyledonous but not in dicotyledonous plants.

Characterization of putative virulence genes from the rice blast pathogen *Magnaporthe oryzae*

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The rice blast fungus *Magnaporthe oryzae* is the economically most important rice pathogen worldwide. While high yield losses in rice cultivation already severely affected global food security, the situation is becoming even worse because a lineage of the pathogen population is threatening wheat and barley cultivation. To fight against the disease, an in depth understanding of the infection mechanism of the fungus is mandatory. To infect plants, M. oryzae secretes a huge number of proteins encoded by virulence genes, which collectively referred to effectors and which manipulate the immune response of the host plant. Here, we report on different putative virulence genes, which are expressed either during the biotrophic or necrotrophic stage of the pathogen´s life-cycle. Candidate genes were selected based on proteome analyses of proteins secreted during the germination of conidia. These data were compared to results of microarray-experiments and with published data on genes reported to be only present in plant associated microorganism. These analyses lead us to a limited number of candidate genes encoding e.g. for pectate-lyase, a jacalin-domain containing protein and nudix-hydrolases. To further characterize these candidate genes, overexpressing and loss-of-function mutants of *M. oryzae* isolate Guy11ku80 were generated and checked for virulence. For localisation studies, genes were tagged with mRFP and constitutively expressed in the fungus or transiently in plants.

Deletion of the putative histone methyltransferase SrSET-2 leads to filamentous growth in *Sporisorium reilianum* f. sp. *zeae*

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The biotrophic fungus *Sporisorium reilianum* occurs in two formae speciales causing head smut on sorghum (f. sp. *reilianum* (SRS)) and maize (f. sp. *zeae* (SRZ)). Although, both SRS and SRZ infect the non-preferred host plant, they do not develop spores there. SRS and SRZ have a high gene identity; however, the gene expression profiles during infection of maize and sorghum are largely diverged. Such differences in global gene expression often depend on epigenetic regulations such as histone methylation. Therefore, we searched the genome of SRZ for putative histone methyltransferases. We found a gene with similarity to SET-2 of *Neurospora crassa*, that we tentatively named SrSET-2. We created SRZ deletion strains lacking SrSET-2 which were verified via Southern blot. Haploid SrSET-2 deletion strains had an altered morphology and grew in short filaments. Despite this morphology alteration, compatible haploid deletion strains were able to penetrate inoculated maize tissue, spread through the infected plant and form spores in the inflorescence. Infection with SrSET-2 deletion strains lead to a stunted growth of the maize plant which showed a correlation to the occurring disease severity. These results indicate, that the putative histone methyltransferase SrSET-2 influences cell morphology and virulence associated genes. Further studies will comprise the verification of the methyltransferase activity of SrSET-2 via in vitro histone methylation assay followed by Western blot analysis.

Genes of *Sporisorium reilianum* cluster 7_11 contribute to host-specific suppression of plant defense in sorghum

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Sporisorium reilianum is a biotrophic smut fungus and two formae speciales exist: *S. reilianum* f. sp. *zeae* (SRZ) and *S. reilianum* f. sp. *reilianum* (SRS). SRS is causing head smut of sorghum and SRZ of maize. Both SRS and SRZ can infect the non-compatible host but will not produce spores. Genome comparison showed that genes of SRS and SRZ share high sequence identity. We created haploid mixed variety segregants (SRSZ) by crossing compatible SRS and SRZ strains, and tested them for virulence on sorghum after mating with a compatible SRS strain. We sorted the SRSZ strains into three virulence groups (fully virulent, intermediate, and non-virulent), and sequenced 188 strains of all three groups. Genome comparison revealed a region on chromosome 7 whose parental origin significantly associated with virulence on sorghum. This region contains the previously identified diversity cluster 7_11 that contains nine genes encoding small putatively secreted proteins whose expression is highly induced during sorghum infection by SRS and SRZ. Deletion of this cluster in SRS leads to phytoalexin formation in sorghum – a defense response observed when sorghum is infected with SRZ. Although this defense response is linked to a proliferation stop of SRZ in sorghum, the deletion strains survived and were able to reach the nodes. Analysis of the effect of cluster deletion on disease incidence on sorghum is currently in progress. We hypothesize that at least one of the nine cluster genes plays a role in host-specific suppression of plant defense in sorghum.

Influence of localization on the function of the *Sporisorium reilianum* effector "Suppressor of Apical Dominance 1"

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Sporisorium reilianum is a member of the phytopathogenic group of smut fungi and able to infect both, *Zea mays* and *Sorghum bicolor*. Infected maize plants show, besides replacement of the inflorescences with teliospores-filled sori, a higher number of subapical ears. The effector protein SAD1 was previously identified to be responsible for the loss of apical dominance in maize after infection, as well as for early inflorescence branching in transgenic *Arabidopsis thaliana*. It was previously shown that SAD1 localizes in the nucleus as well as in the cytoplasm of plant cells. To know which localization is relevant for function, we evaluate the time point of branching in transgenic *A. thaliana* lines expressing SAD1 fused with different localization signals. In addition SAD1 is known to interact with the RING E3 ubiquitin ligase ZmRGLG2. Using Yeast Two-Hybrid analysis we tested the interaction between SAD1 and different domains of ZmRGLG2 as well as AtRGLG1 and AtRGLG2. The N-terminus of ZmRGLG2 could not interact with SAD1 while both the RING and the Copine-domain could strongly interact. Both *A. thaliana* homologs were able to interact with SAD1, with the interaction of AtRGLG1 being weaker than for AtRGLG2. This indicates that SAD1 might function through interaction with RGLG2 and RGLG1 in both maize and Arabidopsis.

Unraveling the role of secreted effectors in complex phyllosphere microbial communities

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To facilitate co-habitation of resource-limited niches microbes have evolved several mechanisms to collaborate or compete with others. Key factors to establish stable microbial networks are so-called microbial hubs. In the *Arabidopsis thaliana* phyllosphere, the oomycete pathogen *Albugo laibachii* has been identified as a central hub, as well as basidiomycete yeasts. One of these yeasts that have been isolated from wild Arabidopsis plants is *Moesziomyces*, which is closely related to the plant pathogenic smut fungi. *Moesziomyces* inhibits several bacterial members of the *Arabidopsis* phyllosphere and suppresses the infection of *A. laibachii*, which identifies this yeast is an important factor for stabilization of the Arabidopsis leaf microbial community. To substantiate this hypothesis we established a high-quality annotated genome sequence and an efficient transformation system. Via RNA-Sequencing we identified genes, mostly glycoside hydrolases and peptidases, and secondary metabolite clusters being upregulated in microbe-microbe interactions. In particular candidate genes being crucial for the antagonistic interaction with *Albugo laibachii* are tested in a reverse genomics approach to identify factors that shape complex microbial communities.

De- and re-construction of fungal virulence

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Fungal pathogens are well-known producers of so-called effector molecules. During infection, fungi have been shown to secrete effectors to facilitate disease. As pathogens rely on effectors to for example manipulate host physiology in order to infect successfully, a better understanding of the modes-of-action employed by different effectors is of high importance. Utilizing the model organism *Ustilago maydis*, causal agent of common smut on corn, enables us to approach effector characterization on a broad scale. By step-wise deconstruction of its armory, we will be able to deprive the fungus of its entire effector repertoire and end up with a non-pathogenic chassis strain. As other obligate biotrophic fungi such as rusts or powdery mildew are not amenable to reverse genetics, we will be able to utilize this empty *U. maydis* chassis to identify and characterize defined sets of effectors of obligate biotrophic fungi.

Cysteine proteases and their inhibitors in microbe-maize root interactions

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Plants are associated with a broad spectrum of microbes and the outcome in plant-microbe interactions ranges from beneficial symbiosis to destructive diseases. The plant apoplast plays a crucial role during the establishment of a plant-microbe interaction. Plant proteases are key players in microbe perception and cysteine proteases belong to the most abundant proteins in the plant apoplast. Among them, papain-like cysteine proteases (PLCPs) have been identified as pivotal components during plant immunity. We propose that regulation of PLCP activity might be necessary to establish an interaction between plant and microbes thus microorganisms need to overcome plant defense responses by modulating, inhibiting or activating, PLCP activity. We have identified a novel root specific PLCP in maize called CP1C due to its high sequence homology to maize CP1A and CP1B. Additionally we found three maize root PLCPs, which are activated upon salicylic acid (SA) treatment,

suggesting a role related to SA-mediated defense responses. Preliminary results suggest that maize endophytic bacteria of the classes Actinomycetes and Flavobacteriia can inhibit maize root PLCPs. Further experiments aim to reveal the microbial molecule responsible for the observed inhibition. Besides, a biochemical characterization of CP1C as well as colonization assays of maize CRISPR-CAS PLCP mutant lines using a maize-SynCom are ongoing experiments to understand the role of PLCPs in maize roots during plant-microbe interactions.

Effector repertoire evolution in smut pathogens

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Smuts are host-specific pathogens mainly on grass species including important agricultural crops, such as maize, barley, oats, wheat and sugarcane. Host colonization and symptom development in these pathogens are facilitated through the secretion of proteins, so-called effectors. The antagonistic nature of the interaction between plants and their pathogens makes that effectors evolve rapidly and are often exemplar for biological innovation. We classified the effector genes of the model smut *Ustilago maydis* in different classes based on various properties including their conservation in other Ustilaginales species and expression pattern in different infection stages. This classification will guide our approach in effector gene deletions to achieve the gradual virulence de-construction of *U. maydis*. Furthermore, we want to elucidate the evolutionary mechanisms that facilitate the rapid effector repertoire diversification. We will perform comparative genomics to study alterations in genome architecture and the relation of these changes to effector repertoire evolution. We will focus especially on the evolution of two Ustilaginales leaf-stripe smut species complexes, *Ustilago striiformis* and *Ustilago serpens*. Species of these complexes only recently diverged from each other, yet appeared to already have evolved host-specific targets as species infect distinct host species. It is intriguing how these pathogens, which generally evolve towards host specific targets, could expand their host range over such small evolutionary timescale. We attempt to reveal the evolutionary mechanisms that enabled the adaptive flexibility for leaf-stripe smuts to shift hosts.

Elucidating the lifestyle of the basidiomycete yeast *Moesziomyces* sp.

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The order of Ustilaginales comprises pathogenic fungi of many important crop plants, for e.g. corn smut caused by *Ustilago maydis*. Besides the plant pathogenic species, anamorphic yeasts have been identified, which form a monophyletic group with the smuts. One example is *Moesziomyces* sp., which has been found on *Arabidopsis thaliana* (Agler et al., 2016) and has no pathogenic sexual morph (Kruse et al., 2017). Given the close association of *Moesziomyces* sp. to *Moesziomyces bullatus*, a pathogen of millet, we asked whether this yeast has the potential do develop infection structures. To this end, a

transgenic self-compatible strain (CB1) of *Moesziomyces* sp. has been generated by stable genomic integration of mating type genes of the *Ustilago hordei*, the causal agent of barley covered smut. Strikingly, the transgenic, self-compatible *Moesziomyces* sp. can be induced to form filaments and appressoria in vitro. When syringe inoculated in two types of millet seeds, we also observed CB1 filamentous growth on the leaf surface. Currently, coleoptile infection assays of millet seeds are tested. Determining a potential host plant for *Moesziomyces* sp. will be an important step to uncover the life style of leaf colonizing basidiomycete yeasts in an ecological and evolutionary context. In addition, we want to investigate the impact of *Moesziomyces* sp. life-style switch from yeast to filamentous growth for its role in the leaf microbial community. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, et al. 2016.. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. PLOS Biology 14(1): e1002352 Kruse J., Doehlemann G., Kemen E., and Thines M. 2017. Asexual and sexual morphs of *Moesziomyces* revisited. IMA Fungus, 8, 117–129.

Identification of *U. maydis* effectors targeting components of quantitative disease resistance in maize

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The biotrophic pathogen *Ustilago maydis* causes smut disease on maize and induces the formation of tumours on all aerial parts of the plant. Unlike in other biotrophic interactions, no gene-for-gene interactions have been identified in the maize – *U. maydis* pathosystem. Thus, resistance of maize to *U. maydis* is considered a polygenic, quantitative trait [1]. However, the molecular mechanisms of quantitative disease resistance in maize and how *U. maydis* interferes with its components is still mostly unknown. Here, we aim to identify *U. maydis* effectors that target maize QTLs. We first assessed *U. maydis* resistance levels in seedlings of the 26 inbred founder lines of the NAM population. Within this diverse set of maize lines, resistance levels ranged from highly susceptible to highly resistant (>94% vs. <35% tumors and dead plants, respectively). An RNA-seq analysis of six *U. maydis*-infected maize lines of diverging resistance levels revealed differential expression of 434 fungal genes, of which 76 are predicted to encode effectors. Next, we generated *U. maydis* CRISPR/Cas9-KO mutants for selected candidate effector sets. Infections of different maize lines with the generated mutants suggest that four of the effectors may have line-specific virulence functions. Taken together, this study not only shows that U. maydis gene expression is dependent on the colonized maize line, but also that *U. maydis* effectors could have a maize line-specific contribution to virulence. Our functional characterization of these maize line-specific effectors will provide new insights into the molecular mechanisms underlying the maize – *U. maydis* interaction. [1] Brefort T, Doehlemann G, Mendoza-Mendoza A, Reissmann S, Djamei A, Kahmann R. *Ustilago maydis* as a Pathogen. Annu Rev Phytopathol, 2009. 47:423–445.

Barley RIC157 is involved in RACB-mediated susceptibility to powdery mildew

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Biotrophic fungal pathogens like *Blumeria graminis* f.sp. *hordei* (Bgh) take advantage of certain cellular host processes in order to successfully establish an infection. Barley RACB, a small monomeric G-protein (ROP, RHO of plants), is required for full susceptibility to penetration by Bgh, potentially due to its role in cell polarization and reorganization of the cytoskeleton. However, since RACB acts basically as a molecular switch transferring signals to downstream interactors, the exact mode-of-action of RACB-mediated susceptibility remains unknown. RIC proteins, a specific class of adapters, have been previously described to interact only with activated ROPs via a conserved CRIB domain and are considered to link ROPs to diverse downstream targets. In the present work we describe a yet uncharacterized RIC protein, RIC157, that interacts in yeast and *in planta* directly with RACB. Interestingly, RIC157 undergoes a recruitment to the cell periphery and plasma membrane in the presence of the activated form of RACB. Transient overexpression of RIC157 rendered barley epidermal cells more susceptible to penetration by Bgh in a RACB-dependent manner, since this elevated susceptibility is abolished by simultaneous transient induced gene silencing of RACB. Preliminary data suggest a role for RIC157 in microtubule organization, as transient overexpression of RIC157 leads to a higher disorder of cortical microtubules. Together with published data, this might indicate that RIC157 is involved in effector-triggered and RACB-mediated support of fungal entry into barley epidermal cells.

Regulation of RAC/ROPs by guanine nucleotide exchange factors in the interaction of barley with the powdery mildew fungus *Blumeria graminis* f.sp. *hordei*

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Successful strategies for genetic crop protection can interfere with signal pathways between the cell surface and host susceptibility factors. RAC/ROP GTPases are molecular switches and play a major role in the interaction of domesticated barley (*Hordeum vulgare*) with the powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (Bgh). RACB is one of these barley GTPases and has been shown to be a susceptibility factor in the interaction since overexpression of activated RACB supports fungal penetration into host epidermis cells, whereas knock-down makes barley more resistant to the pathogen. Guanine nucleotide exchange factors (GEFs) regulate the activity of small GTPases by facilitating the dissociation of GDP and the subsequent binding of GTP to render the molecular switch into an active signalling state. This activating role of GEFs makes them possible regulators of the interaction between barley and Bgh and additionally links RACB with possible upstream cell surface components of the susceptibility pathway. In this work, we study the function of epidermis expressed and Bgh regulated GEFs in barley and their role in the RACB-dependent susceptibility mechanism. First results support GEF-mediated activation of RACB in susceptibility to Bgh and hence RACB-GEFs present possible alternative targets for genetic plant protection.

ROP INTERACTIVE PARTENER b (RIPb) acts downstream of the susceptibility factor RACB and influences the interaction of barley and *Blumeria graminis* f.sp. *hordei*

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RACB belongs to the Rho of plants (ROP) class of small monomeric G-proteins and is a susceptibility factor in the interaction of barley (*Hordeum vulgare*) and the barley powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (Bgh). Over-expression of constitutively activated RACB renders plants more susceptible to Bgh whereas RNAi silencing of RACB decreases plants' susceptibility. ROPs work as molecular switches in many developmental processes, with an active GTP-bound stage and an inactive GDP-bound stage. RACB-silenced plants show defects in root-hair initiation and outgrowth as well as the development of stomatal subsidiary cells, indicating a role for RACB in polar cell development. Since establishment of the fungal haustorium, a specialized cell for nutrient uptake, is accompanied by polar focal ingrowth of the host-derived extrahaustorial membrane, we assume that Bgh exploits a developmental machinery of the host for haustorial accommodation. In order to decipher this process we were seeking for downstream interactors of RACB and found that ROP INTERACTIVE PARTNER b (RIPb) interacts with the activated form of RACB and possibly works as a scaffold protein that mediates RACB signaling to downstream executors. Over-expression of RIPb in single epidermal cells of barley leaves increases the penetration efficiency of Bgh into these cells and Bimolecular Fluorescence Complementation shows that, RACB and RIPb interact at the plasma membrane and on microtubules.. Additionally co-expression of RACB and RIPb show co-localization of the two proteins at the site of fungal attack at early points in the interaction. Therefore, we hypothesize that RIPb acts downstream of RACB in susceptibility to Bgh.

Novel insights into the ligand-receptor pair SCFE1/PCFE1 and RLP30

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Arabidopsis thaliana has evolved a large number of transmembrane cell surface receptors that play major roles during development and are important to respond to external stimuli. We have associated one Arabidopsis leucine-rich repeat (LRR) receptor-like protein, RLP30, with a specific function as pattern recognition receptor for the elicitor SCFE1 (*Sclerotinia* culture filtrate elicitor 1) (Zhang et al., 2013). RLP30 lacks an intracellular kinase domain and therefore associates with the adaptor kinase SOBIR1 for signaling, and additionally recruits the kinase BAK1 after ligand binding (Albert et al., 2015). SCFE1-insensitive Arabidopsis accessions have revealed important regions in the RLP30 receptor, which are most likely implicated in ligand binding or interaction with SOBIR1. SCFE1 promotes typical MAMP (microbe associated molecular pattern) - induced defense responses in Arabidopsis, similarly to the novel elicitor PCFE1 (*Pseudomonas* culture filtrate elicitor 1) from different Pseudomonas strains. Biochemical analyses revealed that both elicitors share the same properties and even more strikingly, are both recognized by RLP30. We believe that SCFE1 and PCFE1 share at least the same minimal binding motif, but more likely, the elicitor activity is derived from homologous proteins. So far, this is one of the very few examples of an elicitor that occurs across different kingdoms.

Chloroplastic ROS and metabolite signalling confer improved resistance to *Colletotrichum higginsianum* in Arabidopsis

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Arabidopsis plants overexpressing peroxisomal glycolate oxidase (GO) in chloroplasts have been established as an elegant system to generate H_2O_2 specifically in a spatiotemporal fashion. In GO plants, glycolate produced during photorespiratory oxygenation of ribulose-1,5-bisphosphate (RubP) by RubisCO is oxidized to glyoxylate directly in the stroma, generating H_2O_2 as a by-product. The amount of H_2O_2 produced in the GO transgenics can be controlled by adjusting the rate of photorespiration by either increasing light intensity, or by lowering ambient CO_2 concentration. By additional overexpression of malate synthase in GO-MS transgenics, the reactive product glyoxylate is converted into malate, thereby preventing its accumulation in the stroma. GO-MS plants behave like GO plants, indicating that H_2O_2 and not glyoxylate is responsible for the responses observed. Similarly, photorespiratory mutants deficient in the major peroxisomal catalase *CAT2* can be triggered to accumulate of H_2O_2 in peroxisomes by the same stimuli. Transcriptome analyses of GO plants and *cat2* mutants revealed a set of genes that are specifically regulated in response to a burst in stromal H_2O_2 in GO plants. These genes include transcription factors *MYB51*, *WRKY33* and *WRKY40* that control the biosynthesis of indolic phytoalexins like indole glucosinolates and camalexin, as well as the transcription factor *ERF6*, which regulates the response of nuclear gene expression to acute oxidative stress in chloroplasts.

We have investigated if and how the signaling processes triggered by H_2O_2 production in response to light shifts and the concomitant induction of indole phytoalexin biosynthesis in GO5 and *cat2-2* affect susceptibility towards the hemibiotrophic fungus *Colletotrichum higginsianum.* We found that the accumulation of the phytoalexin camalexin and the defense messenger salicylic acid were comparable between GO genotypes and *cat2-2* upon pathogen challenge, while ROS accumulation was highest in *cat2-2*. Compared to wild type, GO5 showed improved resistance after light shift-mediated production of H_2O_2, while *cat2-2* became more susceptible and allowed significantly more pathogen entry. Glutathione pool size was increased in both GO5 and *cat2-2*, but unlike GO5, *cat2-2* suffered from severe oxidative stress after light shifts, as indicated by an overall glutathione oxidation state of around 25%.

The complete genome sequence of the host specific *Botrytis fabae* shows distinct differences to the closely related grey mold fungus *B. cinerea* and reveals evidences for their contrasting host ranges

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Botrytis cinerea causes grey mould rot on hundreds of different plant species. In contrast, its closest relative, *Botrytis fabae*, is a host-specific pathogen mostly found on *Vicia faba* only. A gap-less genome sequence of *B. fabae* strain G12 was generated, which had a total length of 45.0 Mb compared to 42.63 *of B. cinerea* strain G12. The increased size was due to the enrichment of AT-rich regions in the *B. fabae* genome, which were devoid of genes. Comparison of the annotated genomes revealed 97.5% nucleotide identity of the orthologous genes between both species, and >10,000 of the 11,422 *B. fabae* genes encoded near-identical proteins (≤5% size difference). Ca. 200 genes were found to occur only in *B. cinerea*, but only 37 genes were present only in *B. fabae*. Furthermore, 600 genes present in *B. cinerea* were found to be >10% truncated in *B. fabae*, whereas only 200 *B. fabae* genes were truncated *in B. cinerea*. Compared to *B. cinerea*, *B. fabae* lacked the genes for 10 secondary metabolite key enzymes, 21 Cyt P450 enzymes, 11 drug efflux transporters, 25 transription factors and 31 secreted proteins. Our data indicate that the evolution of *B. fabae* towards a host-specific infection mode was accompanied by substantial gene loss and gene degeneration, thereby possibly narrowing its physiological versatility.

New tools and markers for improved mutagenesis of *Botrytis cinerea*

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The gray mold fungus *Botrytis cinerea* causes substantial economic crop losses worldwide. For necrotrophic infection, *B. cinerea* secretes multiple proteins and metabolites to kill the host plant. Many of these proteins are known to be involved in the degradation of different plant structures, and/or to display necrotizing activity. To understand the role of this “protein cocktail”, the aim of this work is the deconstruction of the phytotoxic secretome of *B. cinerea*. In a first step, single KO mutants of important toxic proteins using different selection markers were generated. As expected we could obtain wild type-like behaviour in the infection. Based on these single mutants, a multiple KO strategy has been initiated to generate mutants lacking different groups of toxic proteins, to highlight their role in the infection process. Until now, the number of available selection markers in filamentous fungi is limited. We are trying to establish rarely used resistance markers and have developed cyprodinil resistance with a resistant version of the *Bcbos5* gene as a new selection marker for *B. cinerea*.

Molecular characterization of the phytotoxic protein Hrp1 from *Botrytis cinerea*

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Necrotrophic fungi secrete a cocktail of toxins, cell wall-degrading enzymes and phytotoxins during infection. Application of isolated secreted proteins from *B. cinerea* on plant tissue causes massive necrosis within hours. MS-MS analysis revealed more than 200 different proteins within this secretome. Using native 2-D separation, the number of putative toxic proteins could be reduced to 120. Subsequently, 37 promising genes were selected to test for toxicity by transient expression in *N. benthamiana*. This screening allowed to identify novel unknown toxic proteins, from which the Hypersensitive response-inducing protein 1 (BcHrp1) was chosen for further characterization. This protein remains toxic after heat treatment. To pinpoint a minimal amino acid sequence required for toxicity, different truncations of the BcHrp1 were tested in transient expression. However, only full-

length BcHrp1 and none of the truncations caused necrosis after agroinfiltration of tobacco leaves. In addition, tryptic digestion of BcHrp1, resulting in a 66 aa peptide, was also insufficient to maintain toxicity. This indicates that the correct 3D structure of the protein is necessary to confer toxicity. To test whether BcHrp1 is recognized by plant receptors, Arabidopsis receptor mutants (bak1-4 and sobir1-12) where infiltrated with BcHrp1. Preliminary experiments indicated that the mutants showed no lower sensitivity than the Col-0 wild type, suggesting that plant defence against BcHrp1 does not rely on these receptors.

Establishment of CRISPR- Cas in *Botrytis cinerea*

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The gray mold fungus *Botrytis cinerea* is one of the most important pathogens worldwide due to its enormous host range. We used different CRISPR/Cas9 approaches to improve the editing efficiency of *B. cinerea*. Stable or transient genetic delivery of Cas9 together with *in vitro* transfected gRNA was compared with transfection of ribonucleoprotein complexes (RNP). While all approaches resulted in editing, RNP-mediated transformation was found to result in high and most stable editing efficiency. *Bcbos1* encoding a histidine kinase upstream of the Hog1 MAP kinase pathway was used for positive selection of mutants (showing iprodione/ fludioxonil resistance) resulting from Cas9-gRNA mediated cleavage and different repair pathways. We observed highly efficient non-homologous end joining (NHEJ)-mediated error-prone repair, creating predominantly 1-2 bp insertions or deletions. Using a fenhexamid resistance cassette with different sized *Bcbos1* homology flanks, we determined the relative frequencies of NHEJ, microhomology-mediated end joining (MMEJ) and homologous recombination (HR). PCR-generated homology flanks of 60 bp were sufficient for efficient MMEJ-mediated gene replacement, which significantly facilitates and improves marker-based CRISPR/Cas9 genome editing in *B. cinerea*. We are currently trying to establish multiple gene KO mutagenesis and marker-free editing in *B. cinerea*, to overcome the limitation of marker-based genome editing.

Aggressiveness of *Fusarium culmorum* under suppressed trichothecene biosynthesis

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Fusarium Head Blight remains one of the most serious diseases in agriculture. It affects a wide range of crops, spreading fast and contaminating food and forage with poisonous mycotoxins. The interaction between *Fusarium* spp. and plant hosts is extremely complicated and apparently varies from crop to crop. Trichothecene mycotoxins, produced in abundance by these fungi, probably play an important role in disease development and spread. Mutants lacking the ability to produce trichothecenes are less aggressive on wheat, maize, triticale, rye, and barley. This led to the hypothesis that mycotoxins can be used by fungi as tools to weaken and/or slow down plant defenses, making it easier for the pathogen to exploit the host. We tested the aggressiveness of *Fusarium culmorum* when

decreasing the level of trichothecenes produced. We used RNAi to reduce the expression of the tri5 gene. tri5 encodes a trichodiene synthase that catalyzes the first step in trichothecene biosynthesis. Various concentrations of dsRNA of a tri5 fragment were applied to the fungus in vitro. 0.048 µg/mL was the optimal concentration in liquid culture. It decreased tri5 expression by 98 %, leading to reductions in DON and 3-A-DON levels of 85 to 92 %. Correlations between gene expression and concentrations of DON and 3-A-DON were 0.70 and 0.82. For in planta tests, detached wheat leaves of the genotypes “Zlata” and “Ivolga” were sprayed with dsTRI5RNA and point inoculated with F. culmorum conidia. qPCR showed fungal growth to be reduced by 75 % on treated leaves of “Ivolga” and by 50 % on “Zlata”. Gene silencing was 78 and 84 %, respectively. In conclusion, suppression of DON and 3-A-DON production via silencing of tri5 negatively affects *F. culmorum* development on spring wheat varieties. We also found a tendency of fungal growth suppression on wheat by virus-induced gene silencing (VIGS), using barley stripe mosaic virus as viral vector. However, these results were inconclusive so far and to confirm the significance of trichothecenes in wheat susceptibility further experiments are required.