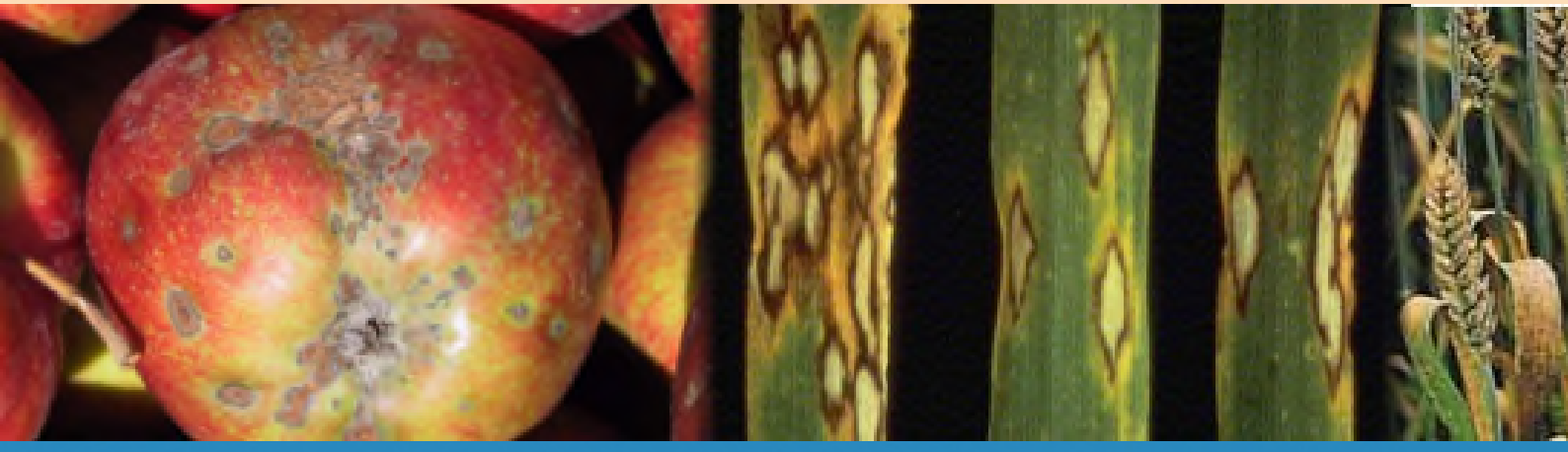
DPG | Spectrum Phytomedizin

Matthias Hahn / Marco Thines (Hrsg.)

57. Jahrestagung des DPG-Arbeitskreises Mykologie und 41. Jahrestagung des DPG-Arbeitskreises Wirt-Parasit-Beziehungen 2022 – Online-Tagung



Zusammenfassungen der Arbeitskreisbeiträge

PI (Persistent Identifier): urn:nbn:de:0294-jb-ak-2022-wpb-0

Deutsche Phytomedizinische Gesellschaft e.V.

Matthias Hahn / Marco Thines (Hrsg.)

57. Jahrestagung des DPG-Arbeitskreises Mykologie

41. Jahrestagung des DPG-Arbeitskreises Wirt-Parasit-Beziehungen

2022

Zusammenfassungen der Arbeitskreisbeiträge

10./11. März 2022

Online-Tagung

PI (Persistent Identifier): urn:nbn:de:0294-jb-ak-2022-wpb-0

Arbeitskreise ‚Mykologie' und ‚Wirt-Parasit-Beziehungen' 2022
Die gemeinsame Tagung der Arbeitskreise ‚Mykologie' und ‚Wirt-Parasit-Beziehungen' fand am 10./11. März 2022 pandemiebedingt online statt.

Die nächste Tagung ist für den 16. / 17. März 2023 an der Technischen Universität München geplant.

Die Zusammenfassungen der meisten Beiträge werden - mit Einverständnis der Vortragenden - im Folgenden wiedergegeben.

Leiter Arbeitskreis Wirt-Parasit-Beziehungen: Matthias HAHN, Kaiserslautern

Leiter Arbeitskreis Mykologie: Marco THINES, Frankfurt

Satztechnische Bearbeitung: Christian Carstensen,
Deutsche Phytomedizinische Gesellschaft e. V.

Tagung der beiden Arbeitskreise Wirt-Parasit-Beziehungen und Mykologie der Deutschen Phytomedizinischen Gesellschaft (DPG) als digitale-Videokonferenz, am 10. und 11. März 2022.

Wie im vergangenen Jahr fand die Jahrestagung auch in diesem Jahr online statt. Erneut wurde das virtuelle Setting von unserem Kollegen Professor Ulrich Schaffrath von der RWTH Aachen organisiert. Ihm gebührt herzlicher Dank für den reibungslosen Ablauf der Tagung. Auch in diesem Jahr wurde die Tagung sehr gut aufgenommen und zeitweilig wurden die Vorträge von mehr als 120 Personen verfolgt. Da uns in diesem Jahr eine außergewöhnlich hohe Anzahl von Beiträgen vorlag, gab es in der diesjährigen Tagung neben den Vorträgen zwei Zeitfenster mit Poster-Pitches. Im Anschluss an diese wurden die Poster in kleinen virtuellen Räumen besprochen, wodurch es zu einem regen wissenschaftlichen Austausch kam. Die Vorträge deckten das gesamte Spektrum der Wirt-Pathogen-Interaktion und phytopathologischen Mykologie ab und zeigten einmal mehr, das ausgezeichnete Forschungsniveau innerhalb der Arbeitskreise. Am Ende der Tagung wurde Herr Prof. Dr. Marco Thines mit einstimmiger Wahl als Arbeitskreisleiter des DPG-Arbeitskreises Mykologie bestätigt.

Im kommenden Jahr soll die Veranstaltung nach dreijähriger Pause endlich wieder in Präsenz durchgeführt werden, wofür erneut Prof. Dr. Ralph Hückelhoven als Gastgeber fungieren wird – wir hoffen dass es diesmal endlich klappt! Als Termin ist der 16./17. März 2023 vorgesehen.

Deutsche Phytomedizinische Gesellschaft e.V.

Arbeitskreis Wirt-Parasit-Beziehungen

Leiter: Prof. Dr. Matthias Hahn

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Arbeitskreis Mykologie

Leiter: Prof. Dr. Marco Thines

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22. Februar 2022

Online-Tagung der DPG-Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“ am 10./11. März 2022 via ZOOM-Videokonferenz

Sehr geehrte Damen und Herren, Liebe Kolleginnen und Kollegen,

nachfolgend finden Sie das Programm der Beiträge zum gemeinsamen Online-Treffen der DPG-Arbeitskreise ‚Mykologie‘ und ‚Wirt-Parasit-Beziehungen‘ am 10./11. März 2022.

Die Videokonferenz wird von Ulrich Schaffrath an der RWTH Aachen ausgerichtet und nicht aufgezeichnet; bitte beachten Sie die üblichen Informationen zum Datenschutz bei der Nutzung vom ZOOM.

Es gibt in diesem Jahr wieder eine erfreulich hohe Beteiligung, mit etwa 130 Anmeldungen und 48 Beiträgen. Um alle Beiträge innerhalb von zwei Halbtagen berücksichtigen zu können, haben wir sowohl für den Donnerstagnachmittag als auch den Freitagvormittag zwischen zwei Sitzungen mit Vorträgen jeweils eine Poster Pitch Session geplant. Leider konnten wir nicht alle Beiträge für Vorträge berücksichtigen, und bitten um Ihr Verständnis dafür, dass wir einige Beiträge, vorbehaltlich der Zustimmung der ReferentInnen, in die Poster Pitch Sessions verschoben haben.

Das Zeitfenster für einen Vortrag ist wie bisher 15 min (10-12 min Redezeit, 3-5 min Diskussion). Wir bitten Sie, die Zeit einzuhalten und die Themen der Vorträge gut einzuführen, damit auch Nichtspezialisten davon profitieren. In den Poster Pitch Sessions stellen die AutorInnen ihre Poster zunächst in Form einer **2-minütigen Kurzpräsentation** vor. Wir bitten hierzu um die **Vorab-Zusendung einer aus 2 Folien bestehenden Zusammenfassung der Highlights Ihres Posters als pdf-Dokument bis zum 9. März, an** hahn@biologie.uni-kl.de und schaffrath@bio3.rwth-aachen.de**.** Anschließend werden Sie paarweise auf ‚Breakout rooms‘ verteilt, die von allen Tagungsteilnehmern in der nächsten ca. 30 min nach Wunsch besucht und verlassen werden können. Es steht Ihnen frei, wie Sie dort Ihre Daten präsentieren (als Poster oder Folien).

Obwohl wir auch dieses Mal (noch!) auf die persönlichen Begegnungen verzichten müssen, freuen wir uns auf die Tagung und auf einen interessanten und lebhaften wissenschaftlichen Austausch.

Mit freundlichen Grüßen

Matthias Hahn und Marco Thines

PROGRAMM

Donnerstag, 10.3.2022

13:00 Uhr BEGRÜSSUNG

VORTRAGS-SESSION 1 (Leitung: Marco Thines/ Fatemeh Salmaninezhad)

13:10 Uhr Sebastian Ploch (Univ. Frankfurt): Diversity of *Wilsoniana* causing white blister disease on cultivated and wild Amaranth

13:25 Uhr Tamara Schmey (TU München): Diversity of the fungal pathogen *Alternaria* spp. on wild tomato plants

13:40 Uhr Bilal Ökmen (Univ. Tübingen): A secreted ribonuclease in biotrophic smut fungi induces cell death in plant

13:55 Uhr *Lingyue Han (Univ. Kiel): MicroRNA* (*miRNA*) *1885) triggers plant defense response to Verticillium longisporum by interfering with plant defense pathways (no abstract)*

14:10 Uhr Florencia Casanova (RWTH Aachen): Towards identification of genes determining host-specificity in *Magnaporthe oryzae* isolates

14:25 Uhr Timo Schlemmer (Univ. Hohenheim): Identification and characterization of sRNA profiles derived from transgenically-expressed and exogenously applied dsRNA precursors

14:40 Uhr Armin Djamei (Univ. Bonn): Maize lipoxygenase 3, a target of Rip1, a ROS-burst suppressing effector of *Ustilago maydis*

14:55 – 15:05 Uhr PAUSE

15:05 – 16:00 Uhr POSTER PITCH SESSION 1 (2 min Kurzpräsentationen, gefolgt von Break-out rooms)

1a. Janina Werner (Univ. Köln): Molecular analysis of fungal hybrids

1b. Shivam Chaudhary (Univ. Jena): A gene cluster responsible for host-specificity in *Sporisorium reilianum*

2a. Alicia Fischer (Univ. Marburg) Ubiquitin conjugating enzyme Ubc8 controls metabolism of storage compounds in *Colletotrichum graminicola* conidia and pathogenicity

2b. Anna Rybecky (Univ. Köln): A modular toolkit for recombinant gene expression in *Ustilago maydis*

3a. Behnoush Hosseini (Univ. Hohenheim): Species of the Diaporthe/Phomopsis complex (DPC) in European soybean and establishment of quadruplex Real-Time PCR for diagnosis

3b. Dimitar Douchkov (IPK Gatersleben): Deep phenotyping of microscopic plant-pathogen interactions opens nonhost resistance to genetics and genomics studies

4a. Julia Seufer (Univ. Marburg): Host carbon allocation influences susceptibility of *Arabidopsis thaliana* to *Colletotrichum higginsianum*

4b. Kristina Munzert (Univ. Marburg): Carbohydrate availability and transport modulate susceptibility of *Arabidopsis thaliana* to *Colletotrichum higginsianum*

5a. Carolin Popp (JKI Dossenheim): Sea Buckthorn Dieback in Northern Germany: Fungal Isolations from shoots and roots

5b. Johanna Wesche (Hochschule Osnabrück): Susceptibility of different apple cultivars to blossom-end rot, caused by the European canker fungus (*Neonectria ditissima*)

6a. Fatemeh Salmaninezhad (Shiraz University, Iran): Transformation of *Pythium oligandrum* using CRISPR/Cas9

6b. Fatemeh Salimi (Univ. of Teheran, Iran): Investigating the growth-promoting and stress-alleviating effects of *Fusarium* spp., dominating the endophytome of halotolerant plants around Lake Urmia, Iran

16:00 – 16:10 Uhr PAUSE

VORTRAGS-SESSION 2 (Leitung: Matthias Hahn/ Johana Misas)

16:10 Uhr Daniela Nordzieke (Univ. Göttingen): Spore-type specific chemotropic growth to maize roots determines root infection by the hemibiotrophic pathogen *Colletotrichum graminicola*

16:25 Uhr Mariana Schuster (Univ. Oxford): AgroLux, a tool for studying plant immunity beyond the hypersensitive cell death

16:50 Uhr Matthias Kretschmer (Univ. Vancouver, Canada): *Ustilago maydis*: A sweet tooth with a taste for sour

17:05 Uhr Weiliang Zuo (Univ. Köln): Sts2, a transcriptional activator secreted from *Ustilago maydis* promotes the tumor formation on maize leaves

17:20 Uhr *Luyao Huang (Univ. Köln): The Ustilago maydis effector protein Tte1 targets the maize corepressor TPL2 to trigger leaf tumor formation (no abstract)*

17:35 Uhr Mamoona Khan (Univ. of Bonn_INRES): Manipulation of plant auxin signaling by a cluster of *Ustilago maydis* effectors

17:50 Uhr Jiangzhao Qian (RWTH Aachen): Long non-coding RNAs in the barley powdery mildew fungus

18:05 Uhr Sabine Engel (RWTH Aachen): DNA hypomethylation accompanies defense priming and systemic acquired resistance0

Freitag, 11.3.22

VORTRAGS-SESSION 3 (Leitung: Monika Heupel/ Jessica Arnhold)

8:30 Uhr Theresa Kabakeris (JKI Kleinmachnow): Which factors influence establishment of powdery mildew (*Erysiphe betae*) in sugar beet?

8:45 Uhr Dorottya Simon (DLR Neustadt/Weinstr.): Investigations on hot water treatment for the production of high-quality grapevine propagation material

9:00 Uhr Annika Hoffmann (Leibniz Centre for Agric. Res. (ZALF)): Blowin' in the wind: wind dispersal ability of phytopathogenic Fusarium in a wind tunnel experiment

9:15 Uhr Stefan Thomas (Univ. Hohenheim): FarmingIOS – hyperspectral imaging for non-invasive disease detection to improve efficiency of precision farming

9:30 Uhr Christian Trautmann (Univ. Hohenheim): A UAS (Unmanned Aerial System) based monitoring system for plant diseases in field vegetable cultures

9:45 Uhr Facundo Ispizua Yamati (Inst. f. Zuckerrübenforschg): Cercospora leaf spot disease prediction and monitoring by an integrated approach based on optical sensor and environmental modeling in sugar beet

10:00 Uhr Alexander Beesley (RWTH Aachen): Engineered coumarin biosynthesis enhances crop resilience

10:15 – 10:25 Uhr PAUSE

10:25 – 11:20 Uhr POSTER PITCH SESSION 2 (2 min Kurzpräsentationen, gefolgt von Break-out rooms)

1a. Jakub Rzemieniewski (TU München) Identification of FRP Peptides as Novel Phytocytokines

1b. Daniel Moser (Univ. Köln) Modulation of apoplastic papain-like cysteine proteases by root commensals of Zea mays

2a. Nassim Safari (TU Kaiserslautern) CRISPR/Cas9-mediated multiple mutagenesis of Botrytis cinerea reveals high redundance of phytotoxic proteins for necrotrophic infection

2b. Andrea Passarge (Univ. Köln) Functional characterisation of rust effectors in Ustilago maydis

3a. Stefan Kusch (RWTH Aachen) The dynamic regulation of transposons in the barley powdery mildew fungus

3b. Georgios Saridis (Univ. Köln) Transcriptional regulation of effector genes in smut fungi

4a. Eileen Baranski (RWTH Aachen) Tailoring coumarin biosynthesis in plants

4b. Polina Marchenko (BOKU Wien) Soil-born endophytic fungi antagonize plant-parasitic root-knot nematodes in tomato

5a. Christin Schulz (Univ. Marburg) The Role of the Ustilago maydis GATA transcription factor Nit2 for the successful infection of maize and nitrogen utilization during biotrophy

5b. Yoon Joo Lee (Univ. Köln): Cell-type specificity in the biotrophic interaction of Ustilago maydis and its host plant maize

11:20 – 11:30 Uhr Pause

Freitag, 12.3.22

VORTRAGS-SESSION 4 (Leitung: Ulrich Schaffrath/ Lukas Weiß)

11:30 Uhr Laura Rehneke (Univ. Ulm): Functional analyses of *Serendipita indica* effector candidates in redirecting phytohormone signalling and activating beneficial effects in Arabidopsis

11:45 Uhr *Parvinderdeep Kahlon (TU München): Engineering of the pattern recognition receptor LORE confers resistance to different plant pathogens in tomato (no abstract)*

12:00 Uhr Lukas Weiß (TU München): Characterization of cellular pathways involved in the RACB-mediated susceptibility of barley towards the powdery mildew fungus Blumeria graminis f.sp. hordei

12:15 Uhr Alex Wegner (RWTH Aachen): MoPl1 and MoNudix are required for full virulence of Magnaporthe oryzae

12:30 Uhr Mansi Singh (RWTH Aachen): Cross-kingdom RNA transfer in barley and powdery mildew interaction

12:45 Uhr Jan Hübbers (RWTH Aachen): Interplay of MLO and exocyst complex proteins in localized secretion

13:00 Uhr Iris Eisermann (Sainsbury Lab, Norwich, England): Defining the septin interactome and its function in appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

13:15 Uhr Termin & Ort für Arbeitskreistreffen 2023

13:20 Uhr Wahl des neuen Leiters des Arbeitskreises ‚Mykologie'

Abstracts der Vorträge und Poster (in der Reihenfolge des Programms)

Abstracts der Vorträge: Session 1

Diversity of *Wilsoniana* causing white blister disease on cultivated and wild Amaranth

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Amaranth is considered an emerging but still undercultivated crop which has the potential to improve human health because it is highly nutritious and produces bioactive substances. But with increasing acreage its diseases caused by numerous pathogens will gain importance. One potentially harmful disease is white blister disease (WBD) caused by fungal-like organisms of the genus *Wilsoniana* (*Albuginaceae*, *Albuginales*, *Oomycota*), which members infect various members of the *Caryophyllales*, including economically important crops. Surprisingly, the biodiversity of *Wilsoniana* on the many wild and ornamental species of Amaranth is poorly investigated, with only a few species described so far. Taking advantage of a large collection of herbarium vouchers the species diversity of *Wilsioniana* on *Amaranth* was phylogenetically investigated based on ITS, *cox*2, and LSU regions. In addition, the specimens were microscopically scrutinized for morphological differences. The phylogenetic tree revealed at least six distinct species infecting the seven investigated *Amaranthus* species, excluding the clades formed by reference sequences from previous studies. Interestingly, most amaranth species host their own, highly host-species specific *Wilsoniana* species. This confirms findings from the sister genera *Albugo* and *Pustula*, which cause WBD on *Brassicales* and *Asterales*, namely that white blister pathogens are generally highly specialised, with the notable exception of *Albugo candida*, which causes disease on various members of the *Brassicales*. Thus, this study provides a first indication that wild amaranth, which is often found as weed beside the cultivated plants, is not necessarily the source for WBD of cultivated Amaranth.

Diversity of the fungal pathogen *Alternaria* spp. on wild tomato plants

Schmey Tamara[1], Small C[1], Gamboa S[2], Lindqvist-Kreuze H[2], Hückelhoven R[1], Stam R[1]

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The wild relatives of modern tomato crops can be found in South America. These plants occur in habitats as different as the Andes and the Atacama desert, but are all more or less susceptible to the fungal pathogens of the genus *Alternaria*. *Alternaria* is a very diverse genus of filamentous fungi. On tomato, several species cause early blight, leaf spot, fruit rot and stem canker diseases. The genetic diversity of tomato-infecting *Alternaria* spp. remains relatively unstudied and no information exists about species that infect tomato wild relatives.

We collected *Alternaria*-like infection lesions from the leaves of 8 wild tomato species from Chile and Peru. Using molecular barcoding markers, we characterized the collected pathogens. Morphological observations and an infection assay confirmed the molecular analyses. The infection lesions were caused predominantly by small-spored *Alternaria* from section *Alternaria* like *A. alternata*, but also by *Stemphylium* spp., *Alternaria* spp. from section *Ulocladioides* and further related species. These findings highlight the diversity of the pathogens from this wild plant-pathosystem. Further comparative genomic analyses of these wild isolates will increase our understanding of how this pathogen adapts to host and climate. Seeing that *A. alternata* has been reported to be a growing problem on cultivated tomato, investigating the evolutionary potential of this pathogen is not only interesting to scientists studying wild plant-pathosystems but could inform tomato crop protection and plant breeding programs in the distant future.

A secreted ribonuclease in biotrophic smut fungi induces cell death in plant

Bilal Ökmen[1,2], Raphael Wemhöner[1] and Gunther Doehlemann[1]

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In order to successfully colonize their hosts, fungal pathogens establish a complex network of interactions not only with their respective host but also with other organisms present in the environment. These involve production and secretion of effectors to manipulate host defense and metabolism, and secretion of antimicrobial compounds to modulate host microbiota. Ribotoxins are a group of highly specific extracellular ribonucleases that specifically target and

cleave the universally conserved sarcin-ricin loop sequence of rRNA, which leads to inhibition of protein biosynthesis and subsequently to cell death.

In this study, we have characterized the RNase T1 which is conserved in all smut fungi. Although smut Ribo1 is more closely related to the non-toxic RNase T1 members, heterologous expression of Ribo1 from *U. hordei*, *U. maydis*, and *Sporisorium reilianum* in *Nicotiana benthamiana* revealed cell death-inducing activity. To functionally understand the mode of Ribo1-induced cell death, active site mutants of both secreted and non-secreted version of UhRibo1 have been tested. While extracellularly induced cell death is independent from enzymatic activity, cell death induced by intracellular expression requires an active Ribo1 enzyme. Thus, we hypothesize that while the mode of intracellularly UhRibo1-induced cell death is associated with its rRNA cleavage activity, the extracellular UhRibo1 is most likely recognized by a yet unknown plant receptor to induce defense responses.

Towards identification of genes determining host-specificity in *Magnaporthe oryzae* isolates

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Magnaporthe oryzae causing rice blast disease is the economically most important plant pathogen of rice. Severe epidemics on wheat and barley caused by certain isolates of the fungus have been reported in recent years from South America. A way to elucidate the molecular basis for host preferences is the employment of comparative genomic analysis. Here we present data on two genes that are absent in rice-infecting isolates, but present in others, including wheat-infecting isolates. To disclose if these genes determine host specificity of *M. oryzae* we utilized genetical manipulations and deleted both genes in a wheat-infecting isolate by CRISPR/Cas9 technology. Additionally, these genes were transformed into the rice-infecting isolate *M. oryzae* Guy11. We further started to develop an inducible gene expression system for *M. oryzae*, which would enable to regulate genes during the infection process. This technique is based on a copper-dependent inducible promoter and would expand the molecular tool box for *M. oryzae* functional genomics.

Identification and characterization of sRNA profiles derived from transgenically-expressed and exogenously applied dsRNA precursors

Schlemmer T[1] , Barth P[2] , Koch A[3]

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CYP3RNA is an artificially designed 791-nucleotide-(nt)-long, double-stranded RNA that contains three homologous sequences (~300 nt each) of the CYP51 paralogs (ordered; CYP51B, CYP51A, CYP51C) from Fusarium graminearum (Fg). We previously demonstrated that transgenically- expressed CYP3RNA and exogenously sprayed CYP3RNA confer strong Fg resistance in Arabidopsis thaliana and barley (Hordeum vulgare). Until now, processing of CYP3RNA and its derived small(s)RNA profiles were unexplored. We have now identified sRNA pattern by RNA-sequencing Fg liquid cultures treated with CYP3RNA, Arabidopsis thaliana plants that express the CYP3RNA endogenously, and barley leaves that were sprayed with CYP3RNA. Most sRNAs identified in all three organisms derive from the CYP51A fragment, which is in the middle of the CYP3RNA precursor. In addition, 80% of sRNAs derived by transgene expression are 21-nt long, while the length of sRNAs of exogenously applied CYP3RNA to Fg or barley reveals a double peak at 19 nt and 21 nt. Several hundred sRNAs were identical in all three organisms. Analysis of the sRNA pattern showed a conserved origin of a 100-nt-long sequence in the CYP51A fragment, which we termed Hot-Spot A (HSA). We tested HSA but also rearranged CYP3RNA constructs (CYPABC and CYPBCA) by expressing them in Arabidopsis thanliana plants. All tested constructs conferred Fg disease resistance and provoked target gene silencing as well as co-silencing of the FgCYP51B gene by the HSA-derived sRNAs.

Co-repressor Topless, the central effector hub for *Ustilago maydis* / maize interaction

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The biotrophic fungus *Ustilago maydis* causes smut disease in maize (*Zea mays*) and teosinte *(Zea mays ssp. parviglumis)*. Upon establishment of biotrophy, *U. maydis* secretes manipulative molecules, called effectors, to shape this interaction, to suppress immune responses and to redirect host metabolism and development in favor of the pathogen. Transcriptomic analysis of *U. maydis*-infected maize show changes in several phytohormone signaling pathways, among others an upregulation of auxin and jasmonate signaling during establishment of biotrophy, but the molecular basis of this signaling manipulation was long time unknown.

Here we report our recent findings on several translocated effectors that all target maize Topless (TPL) co-repressor family members and lead upon *in planta* expression to specific de-repression of either Ethylene/Jasmonate signaling or auxin signaling. We demonstrate a direct link between TPL and PAMP-triggered Immunity responses, highlightening the role of TPLs as molecular players in plant defense / growth antagonisms in plants. Furthermore, the sheer number of TPL manipulating effectors implicate an outstanding importance of these effectors to smut fungi, giving many interesting insights both, in plant and pathogen biology.

Abstracts: Poster Pitch Session 1

Molecular analysis of fungal hybrids

Werner J, ZuoW, Depotter JRL, Doehlemann G

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The smuts consist of more than 1,500 species and are highly economically important as they infect relevant crops. Most smuts are known to infect their host systematically and replace the inflorescences with teliospores, e.g. *Sporisorium reilianum*. Unlike other smuts, *Ustilago maydis* infects all aerial parts of the plant and forms large tumors on maize leaves as well as in the inflorescences.

U. maydis and *S. reilianum* are closely related and have similar genomes regarding the synteny and the size. Previously, genome comparison revealed that *U. maydis* and *S. reilianum* possess conserved effector genes even though they differ fundamentally in their pathogenesis on the same host, *Zea mays*. Recently, a fungal hybrid combination of *U. maydis* FB1 and *S. reilianum* SRZ2 was used to investigate fungal hybridization. Storfie and Saville (2021) showed that the alteration in gene expression in the hybrid can alter the virulence, however, the hybrid could not exhibit extensive hyphal growth or progress in pathogenic development.

Thus, we hypothesized that only a change of the mating type genes between the species can lead to a successful hybridization. Due to their genomic synteny, interspecific hybridization of recombinant strains was generated to investigate the impact on virulence and host specificity. RNAseq is used to get further insights in the evolution of the different disease development on gene expression level and genome compatibility.

REFERENCE

Storfie, E.R.M. & Saville, B.J. Fungal Pathogen Emergence: Investigations with an *Ustilago maydis* × *Sporisorium reilianum* Hybrid (2021). Journal of Fungi, 7, 672.

A gene cluster responsible for host-specificity in *Sporisorium reilianum*

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Sporisorium reilianum is a facultative biotrophic smut fungus that causes head smut disease in maize and sorghum. This soil-borne pathogen infects the plants at the seedling stage and leads to a systemic colonization of a susceptible host plant and the generation of fungal spore-

filled sori that partially or completely replace inflorescences. *S. reilianum* exists in two formae speciales, *S. reilianum* f. sp. *reilianum* (SRS) and *S. reilianum* f. sp. *zeae* (SRZ) that infect sorghum and maize, respectively. Both formae speciales can penetrate and multiply in their reciprocal hosts but do not produce spores. At the genomic level, both formae speciales of *S. reilianum* are very similar. To identify host specificity factors, we followed an approach combining classical genetics with next generation sequencing and GWAS analysis. This revealed a region of 35 genes, whose parental origin from the SRS parent is highly correlated with the capacity to form spores on sorghum. Within the region of interest is a gene cluster encoding nine putative effector proteins that are predicted to carry signal peptides and show low amino acid sequence conservation between SRS and SRZ. Cluster genes are upregulated during plant infection. By generation of single gene deletion strains, we could show that at least three of the nine genes contribute to the full virulence capacity of SRS on sorghum.

Ubiquitin conjugating enzyme Ubc8 controls metabolism of storage compounds in *Colletotrichum graminicola* conidia and pathogenicity

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An *Agrobacterium tumefaciens*-mediated insertional mutant collection of the hemibiotrophic ascomycete maize pathogen *Colletotrichum graminicola* was screened for mutants with weakly affected pathogenicity in order to identify genes involved in fungal nutrition. A T-DNA insertion near the Ubc8 homolog of *Colletotrichum graminicola* showed reduced virulence, which could be confirmed in targeted knockouts of *CgUbc8*. In the yeast *Saccharomyces cerevisiae*, the ubiquitin-conjugating enzyme Ubc8 had already been identified to play a central role in regulation of gluconeogenesis through the ubiquitin-dependent degradation of the irreversible enzyme fructose-1,6-bisphosphatase (FBPase).

Our work has revealed that the glycogen content in *C. graminicola* Δubc8 knockout mutants is significantly reduced in young conidia. When glycogen is mobilized during conidia maturation, lipid accumulation was increased in mutant compared to wild type. Hence, our data suggest that CgUbc8 is involved in the regulation of the interconversion and mobilization of reserve compounds during maturation of *C. graminicola* conidia. Pulldown and MS analysis of ubiquitinated proteins from young conidia revealed that several enzymes of glycolysis were less ubiquitinated in Δubc8 knockout mutants than in wild-type and we are currently investigating the contribution of the identified enzymes during the individual stages of conidia

maturation. With the help of a CgUbc8:3xHA *in locus* fusion, we aim to identify direct and indirect CgUbc8 interaction partners *in vivo.* References

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A modular toolkit for recombinant gene expressionin *Ustilago maydis*

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Smut fungi are capable to affect grasses of the Poaceae family, including economically relevant crops such as *Zea mays* (maize). *Ustilago maydis* is a smut fungus, that induces tumour formation in all aerial parts of it host and requires a passage through the plant in order to complete its life cycle. Its biotrophic nature enforces *U. maydis* to establish an intimate relationship with the plant host by secreting a plethora of effector proteins.

Together with the possibility to manipulate it under laboratory conditions and its well established plant-pathogen system, *U. maydis* constitutes a model organism to understand the biology of functional effectors and biotrophic fungal lifestyle. Nevertheless, the large number of effector proteins secreted by *U. maydis* and the potential redundancy in function, complicatesthe study of their role in virulence by knockout approaches.

In this study we generated a Modular Recombinant Fungal toolkit for Gene expression in *U. maydis* (MoRFunG toolkit) that allows efficient gene expression and gene manipulation. The commutable nature of the MoRFunG toolkit, virtually permits not only the creation of tailored made transcript units based on *U. maydis* sequences, but also the expression of effector genes from obligate pathogens under the control of *U. maydis* promoters. The experimental establishment of the MoRFunG modules was applied for the virulence cluster 6A, which comprises 8 effector genes whose contribution for fungal virulens within the cluster have been elucidated.

Species of the *Diaporthe/Phomopsis* complex (DPC) in European soybean and establishment of quadruplex Real-Time PCR for diagnosis

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Phomopsis seed decay is known as one of the most destructive soybean diseases, affecting seed quality and causing massive yield losses worldwide. The disease is caused primarily by *Diaporthe longicolla* along with other DPC species. Precise identification of the species of this complex is necessary for understanding the epidemiology of the disease and for optimal control. Based on the isolation of 32 DPC strains from DPC-damaged European soybean seeds we identified four species: *D*. *longicolla*, *D*. *caulivora*, *D*. *eres* and *D*. *novem*. These four species can be considered the principal DPC species on soybean in Central Europe. We now aim to develop a fast and accurate method to detect these pathogens via quadruplex Real-Time PCR. Based on sequences of translation elongation factor 1-alpha (*TEF1*), four specific TaqMan primer-probe sets were designed and tested for specificity and efficiency using DNA from pure cultures of these species and other important soybean pathogens from the genera *Sclerotinia, Colletotrichum, Fusarium*, *Uromyces*, and *Phakopsora*. Our primer-probe sets allow excellent discrimination of the different DPC species and can be used to detect and distinguish them in parallel using quadruplex Real-Time PCR. The quadruplex assay was tested on different plant material including healthy and infected soybean seeds or seed coats, soybean stems, and leaves. Moreover, the quadruplex Real-Time PCR was adapted to quantify these pathogens relative to the amount of plant material. Standard curves were created from serial dilutions of genomic DNA from each of the pathogens and from soybean plants. To quantify the amount of fungal DNA (ng) per plant DNA (ng) with the help of the standard curves, DNA samples from six soybean seed lots were tested in the quadruplex Real-Time PCR assays and SYBR Green-based Real-Time PCR assays. The results indicated that the amount of fungal biomass seems to be highly variable between individual seeds. We now want to deveolop the assay into a standard procedure for testing soybean seeds, plant material, and soil, and are planning comprehensive sampling to study the epidemiology of DPC species in Germany. Other aspects of our future research will be testing different soybean cultivars for their resistance against the different DPC species. On mRNA level we also want to study soybean responses against the *Diaporthe* species in general.

A deep phenotyping platform for microscopic plant-pathogen interactions opens nonhost resistance to genetics and genomics studies

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Using the powdery mildew fungi of cereals as a model system, we have developed an automated microscopy pipeline coupled with deep learning-based image analysis for high-throughput phenotyping of plant-pathogen interactions. The currently available microscopic phenotypes include fungal microcolony count and density, precise area of the secondary hyphae of each colony, and different morphological parameters. The micro-phenotyping pipeline was coupled with macroscopic imaging in the visible and near-infrared spectra for

establishing a deep phenotyping platform for plant-pathogen interactions. Furthermore, the high throughput and sensitivity of the system allow quantifying rare microscopic phenotypes in a large sample size. One of these phenotypes is the cryptic infection of non-adapted pathogens, marking the hidden transition stages of pathogen adaptation and breaking the nonhost barrier. Thus, our tool opens the nonhost resistance phenomenon to genetics and genomics studies.

Host carbon allocation influences susceptibility of *Arabidopsis thaliana* to *Colletotrichum higginsianum*

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The hemibiotrophic ascomycete fungus *Colletotrichum higginsianum* is adapted to the model plant Arabidopsis. Previous work in our group has shown that *C. higginsianum* does not depend on carbon supply by intact host cells during the biotrophic phase, but that carbohydrate availability and allocation in the host tissue are important determinants for the efficiency of the salicylic acid-mediated defense response.

On one hand, the starch free Arabidopsis mutant *pgm* shows impaired carbohydrate availability and is more susceptible towards *C. higginsianum*. Early post-penetration establishment in starchless *pgm* mutants is enhanced due to altered cell wall composition, while during the transition to the necrotrophic phase, *pgm* exhibits dampened responsiveness of salicylic acid induced genes despite increased levels of free salicylic acid.

On the other hand, the double mutant *sweet11/sweet12,* which lacks the two major phloem loading sucrose transporters, has been shown to be more resistant than the wildtype during pathogen challenge. The *sweet11/12* mutant shows elevated levels of free sugars and salicylic acid as well as increased responsiveness of salicylic acid dependent genes.

We have now studied *sweet11/sweet12/pgm* triple mutants in order to assess the interdependence of these opposing interaction phenotypes. In comparison to *pgm*, the triple mutant exhibited alleviated hypersusceptibility towards *Colletotrichum higginsianum*, which was accompanied by further increased accumulation of soluble sugars compared to the *pgm* parent and further enhanced premature SA accumulation compared to the *sweet11/sweet12* parent. Our data strongly favour the idea that carbohydrate availability and the defence response are increased in the triple mutant compared to *pgm*, because excess sucrose export to roots is prevented. Using the triple mutant as a tool, we currently investigate the crosstalk of sugar and defence signalling, In addition, we are using genetically encoded fluorescent

nanosensors to assess the spatiotemporal organization of the defence response in the epidermis of the individual genotypes.

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Carbohydrate availability and transport modulate susceptibility of *Arabidopsis thaliana* to *Colletotrichum higginsianum*

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Plant carbohydrate availability has profound effects on susceptibility to fungal pathogens. The starch-free *Arabidopsis thaliana* mutant *phosphoglucomutase* (*pgm*) suffers from nocturnal carbohydrate shortage and impaired defence against the hemibiotrophic pathogen *Colletotrichum higginsianum*. Previous studies revealed that both preformed defence like the composition of the plant cell wall as a penetration barrier, and induced defence responses like accumulation of the phytohormone salicylic acid and the phytoalexin camalexin are impaired in *pgm*, leading to hypersusceptibility to *C. higginsianum*. To identify genes involved in the regulation of carbohydrate-dependent pathogen susceptibility, we performed a forward genetic screening and identified suppressors of *pgm* hypersusceptibility to *C. higginsianum*. The selected *pgm* hypersusceptibility suppressor candidates were mapped using bulked segregant analysis followed by whole genome sequencing to identify the causative mutations. Additionally, the candidates were analysed for alterations in their carbohydrate metabolism, cell wall monosaccharide composition as well as differences in their induced defence responses to *C. higginsianum* in comparison to *pgm*. We found that the increased resistance was partially established in the early biotrophic interaction with *C. higginsianum* and correlated with changes in cell wall composition, as well as in the production of defence-related secondary metabolites. One of the suppressor candidates was mapped to *OUTER ENVELOPE PROTEIN 40 (OEP40)*, coding for a b-barrel solute channel in the chloroplast outer envelope membrane. OEP40 is permeable for glucose, glucose 1-phosphate and glucose 6-phosphate and regulated by trehalose 6-phosphate *in vitro*, suggesting a function in sugar homeostasis and/or signalling in the context of pathogen defence.

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Sea Buckthorn Dieback in Northern Germany: Fungal Isolations from shoots and roots

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During recent years, an increased dieback of sea buckthorn plants could be observed in northern Germany. In some areas, plant death occurs to a dramatic extent having an ecological und economic impact, as both wild plants and plantations are affected. Symptoms comprise wilting of single branches with dried-out leaves and fruits, shoots shrinkage, black-reddish discoloration and lesions of the bark, and discolorations in cross sections of the shoot. Up to now, the cause of plant-death remains unknown.

A joint-project, HippRham, started in 2020 to reveal the cause of sea buckthorn dieback and to develop possible control strategies. Extensive isolations were performed as part of pathogen diagnostics. From a total of surface sterilized 172 samples, 1923 shoot and root pieces were placed on malt extract agar for isolation. Of these, 1100 fungal cultures were obtained. Most frequently isolated and only derived from symptomatic shoots were the genera *Hymenopleella* and *Diaporthe*. In contrast, *Aureobasidium*, *Cladosporium*, *Alternaria*, *Epicoccum*, and *Pencillium* were identified from both symptomatic and asymptomatic, neighboring, plants. In roots originating from symptomatic plants *Penicillium* and *Mucor* were most frequently determined, followed by *Ilyonectria*. Inoculation experiments will be set up to investigate the impact of selected fungal isolates on sea buckthorn plants. In addition, a culture-independent sequencing approach is in preparation to obtain an overview of the total sea buckthorn fungal community.

Suceptibility of different apple cultivars to blossom-end rot, caused by the European canker fungus (*Neonectria ditissima*)

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European fruit tree cancer, caused by the phytopathogenic fungus *Neonectria ditissima*, is one of the most economically important diseases of apple in the Lower Elbe Region. *N. ditissima* is a wound parasite and primarily causes the typical canker lesions on wood. In addition, the pathogen can act as a fruit pathogen, causing two different types of rot, viz blossom-end rot and storage rot. Blossom-end rot is due to flower infections and has become more prevalent in recent years. By means of artificial inoculations, Holthusen and Weber (2021) were able to determine the exact time of infection to full bloom (BBCH 65) for the apple cultivar 'Pinova' (Evelina®) and to the period from full bloom to declining bloom (BBCH 67) for 'Nicoter' (Kanzi®). Based on these results, further trials for flowering were set up in 2021.

Cultivars are known to possess susceptibilities to cancer infections in woody tissue. It is assumed that resistance mechanisms are located on the leaf scars of the trees (Amponsah et al. 2015). It is therefore uncertain whether the known susceptibility from the literature can be transferred to blossom-end rot. The aim of this work was to investigate the susceptibility of different cultivars to blossom-end rot caused by *N. ditissima*. First results indicate that the cultivars 'Boskoop' and 'Gravensteiner' showed a significantly higher incidence of infection. This differs in part from the known susceptibility to canker lesions on woody tissue.

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Transformation of *Pythium oligandrum* using CRISPR/Cas9

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Oomycetes are ubiquitous filamentous eukaryotes related to brown algae and diatoms, but unrelated to opistokont fungi. While many members of the groups are devastating plant pathogens, e.g. the potato late blight pathogen, Phytophthora infestans, there are also some species that might have beneficial effects on plants. For example, Pythium oligandrum is an oomycete that can promote plant growth by parasitising antagonistic fungi and oomycetes. These properties have been assumed to be linked to a small secreted protein named oligandrin. To test the potential effect of this protein in the interaction with plants and antagonistic fungi, we conducted an oligandrin gene knock-out experiment using CRISPR/Cas9. Several guide-RNAs for oligandrin and ornithine transferase (as marker) gene knock-out were designed. Neomycin phosphotransferase and eGFP (enhanced green fluorescent protein) were selected as markers for Cas9 expression. The transformation of four stains of P. oligandrum was performed using polyethylene glycol (50 % w/v PEG4000) mediated protoplast transformation. Protoplast regeneration was observed on liquid and solid V8 medium containing 20 µg/mL Uracil, 30 µg/mL Geneticin (G418), and 100 µg/mL Ampicillin. The eGFP expression was checked using fluorescence microscopy. Five of 60 clones expressed both eGFP efficiently and were uracil deficient. In upcoming experiments the performance of strains as growth-promoting and mycoparasitic oomycetes will be tested. To our knowledge, the current study is the first to report a successful attempt to transform a species of Pythium using the CRISPR/Cas9 system.

Investigating the growth-promoting and stress-alleviating effects of *Fusarium* spp., dominating the endophytome of halotolerant plants around Lake Urmia, Iran

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Endophytic fungal strains were recovered from roots, stems and leaves of healthy *Phragmites australis*, *Salicornia* spp., *Atriplex* spp., and *Suaeda* spp. collected from saline soils of 16 different sites of five salinity classes around the Lake Urmia, at East and West Azarbaijan provinces, Iran. A total number of 300 (31%) out of 960 obtained strains were tentatively identified as belonging to the genus *Fusarium* based on morphology. Phylogenetic analyses inferred from ITS and *tef-1α* sequences revealed that the recovered *Fusarium* s.l. strains belong to eight phylogenetic groups (Burgessii, Fujikori, Incarnatum, Nisikadai, Oxysporum, Redolance, Sambucinum Tricintum) and 20 species. *Fusarium proliferatum* was identified as

the dominant and most prevalent endophytic species, isolated from all four plant hosts, all three plant tissues, all geographical sites and all five salinity classes. The growth response of all *Fusarium* strains against 3.5% NaCl and 3.5% KCl was assessed on culture media. The various species tested showed significant differences in terms of growth rate on these extreme conditions. After this, the ability of the endophytic *Fusarium* species to enhance the growth of barely and rice seedlings was assessed. Results varied depending on the fungal species and the host plant**.** *Fusarium gamsii, F. algerience* and *F. culmorum* had a negative effect on rice and barley seedlings, whereas, *F. tricintum, F. redolance, F. toxicum* and *F. pisi* decreased the growth of rice seedlings. In the next step, the potential role of the obtained endophytic *Fusarium* strains of fungi in promoting the growth of barely and rice under salinity stress in a mutualistic manner will be investigated.

Keywords: Ascomycota, Biodiversity, Endophytic fungi, Salinity, Symbiosis

Abstracts der Vorträge: Session 2

Spore-type specific chemotropic growth to maize roots determines root infection by the hemibiotrophic pathogen *Colletotrichum graminicola*

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The hemibiotrophic fungus *Colletotrichum graminicola* is a maize pathogen infecting several plant tissues like leaves, stems, and roots. In the field, disease symptoms like plant stunting are observed early in the season, whereas anthracnose lesions harboring falcate conidia appear at later timepoints. We currently follow the hypothesis that oval conidia, a second asexual spore type formed in the vascular system of infected maize, are causing plant stunting by efficient root infection.

We have recently shown that oval and falcate conidia exhibit specialized leaf infection strategies (Nordzieke et al 2019). To test whether such specialization exists also for the infection of maize roots, we have mimicked the root infection process in the field and planted maize seeds in sporeenriched soil. In this experimental setup, only oval conidia provoked severe stunting of theyoung maize plants, indicating a host recognition process prior to infection. We therefore tested the ability of maize root exudate (MRE) to provoke directed growth of both conidia types using a recently developed 3D printed device combined with a fluorescent marker for polar growth (Schunke et al 2020, Growth et al 2021). Intriguingly, only oval conidia showed a strong chemotropic growth to MRE. As HPLC/MS analyses demonstrate, so far unknown secreted signaling molecules from MRE are responsible for the attraction of *C. graminicola* oval conidia. To verify the impact of such spore-type specific adaptions for maize root infection in the field, we overlayed planted maize seeds with leaves or stems of maize plants suffering a systemic anthracnose infection. Strikingly, we observed *C. graminicola* infection of maize roots only in the samples overlayed with infected stems, the generation points of oval conidia.

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AgroLux, a tool for studying plant immunity beyond the hypersensitive cell death

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Transient co-expression of immune receptors and their matching effector proteins induces effector-triggered immunity (ETI). ETI responses generally include cell death associated with the hypersensitive response (HR). We recently developed AgroLux, a robust and rapid quantitative assay to detect effector-triggered immune responses before tissue collapse. Cell death triggered upon co-expression of Avr genes with matching Cf resistance genes, is preceded by a reduced AgroLux luminescence (Jutras et al, 2021). To unravel the underlying mechanism of reduced AgroLux, we tested known nodes in Avr4/ Cf4 immunity for their role in Avr4/Cf4-triggered reduced Agrolux bioluminescence. We discovered that whereas tissue collapse is always preceded by AgroLux reduction, such reduction also occurs in several mutants that are impaired in Avr4/Cf4-triggered HR. Moreover, we discovered genetic components required for Avr4/Cf4-mediated Agrolux reduction. We will present advances on our understanding the pathway required for Cf4/Avr4 immunity and discuss the significance of reduced AgroLux bioluminescence in these conditions.

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Ustilago maydis: A sweet tooth with a taste for sour

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Ustilago maydis is a biotrophic plant pathogen of corn. Biotrophic plant pathogens are strictly dependent on a host to fulfill their lifecycle. The lack of information regarding nutritional requirements during biotrophy at present is limiting research progress. Here, we show that a combination of preferred and non-preferred carbon sources such as glucose and organic acids leads to three remarkable phenotypes in *Ustilago maydis in vitro*. *Ustilago maydis* grown in minimal medium with glucose and malate showed accelerated growth, the production of an extracellular polysaccharide and the formation of melanin. With RNAseq we identified the activation of the biotrophic program including expression of secreted effectors during growth on mixed carbon sources. A dicarboxylate uptake system was identified and its relevance for fungal virulence was shown. Further, we were able to identify a melanin biosynthesis cluster, which contributes to melanin formation during sporulation. Accelerated growth and partly melanin formation were also seen for other biotrophic and hemibiotrophic fungal plant pathogens. Taken together, acquisition and usage of mixed host derived carbon sources may be a conserved feature of fungal biotrophy.

Sts2, a transcriptional activator secreted from *Ustilago maydis* promotes the tumor formation on maize leaves

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Ustilago maydis causes common smut disease in maize. One of the exclusive phenotypes is its ability to induce localized host cell proliferation upon infection on aerial tissues such as leaf, tassel and ears. However, the underlying mechanism is still poorly understood.

In our previous study, we identified a leaf-specific effector[1], which is significantly higher induced and may evolved a diverse function compare to its orthologs from another close related maize smut pathogen *Sporisorium reilianum*[2]. This effector protein, termed Sts2 (small tumor on seedlings 2),.is translocated into the plant cell nucleus. Sts2 functions as a transcriptional activator and this activity is essential for its virulence function in *U. maydis*, which is required to induce cell divisions of infected

maize bundle sheath cells. Sts2 interacts with ZmNECAP1 (adaptin-ear-binding coat-associated protein 1), another novel plant transcriptional activator. Further study on the role of ZmNECAP1 in leaf development and *U. maydis* triggered tumor formation will help us to understand how the pathogen hijacks the host development process to promote its pathogenic development.

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The *Ustilago maydis* effector protein Tte1 targets the maize corepressor TPL2 to trigger leaf tumor formation

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Plant development and environmental response requires the fine-tuned regulation of gene expression. Activation or repression of gene expression is controlled by transcriptional regulation factors, such as transcription factors, coactivators and corepressors. A well known plant corepressor is encoded by TOPLESS (TPL). The TPL corepressor recruits transcriptional repressors that contain an EAR (Ethylene responsive element binding factor Amphiphilic Repression) motif. The maize smut fungus *Ustilago maydis* has been shown to produces two effector proteins Jsi1 and Naked1, which are involved in the induction of jasmonate/ethylene (JA/ET) and auxin signaling pathways by interactions with TPL corepressors (Darino et al. 2020; Navarrete et al. 2021). In this study, we characterized a *U.maydis* virulence effector TOPLESS target effector 1(Tte1), which is specifically required for the formation of leaf tumors. We found that Tte1 impacts the maize TOPLESS gene ZmTPL2 through interaction with its N-terminal domain via its EAR motif. We observed that the interaction between Tte1 and ZmTPL2 alters the nuclear distribution pattern of ZmTPL2. We propose that Tte1 interferes with oligomerization of ZmTPL2, which results in a re-localization from nuclear speckles towards the cytoplasm. Our findings provide new mechanistic insights into the effector-mediated modulation of plant signaling during pathogen-induced formation of plant tumors.

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Manipulation of plant auxin signaling by a cluster of *Ustilago maydis* effectors

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Biotrophic plant pathogens employ secreted molecules, called effectors to suppress their host immune system and to redirect the metabolism and development in their own favour. The biotrophic fungus *Ustilago maydis* causes smut disease in maize (*Zea mays*) and teosinte (*Zea mays* ssp. *parviglumis*). Its genome encodes approximately 550 predicted secretory proteins that likely function as effectors, of which round one fifth are localized to gene clusters. During plant colonization U. maydis also secretes certain plant growth substances including auxins. The levels of auxin are increased in the infected tissues of maize and auxin signalling and auxin responsive genes are transcriptionally up-regulated. Our understanding how *U. maydis* employs auxin signalling is just recently emerging.

Here we tested the ability of *U. maydis* putative effectors to induce growth hormone auxin signalling in plants and identified a set of five, genetically clustered effectors. By using different cell biological and biochemical approaches we identified central corepressors of the Topless family as targets of these effectors. We demonstrate that the auxin signalling inducing subcluster effectors play a role in virulence. Topless Interacting protein effectors (Tips) interacts solely with the N-terminal TPD domain and compete with Aux/IAA transcriptional repressors for their binding. Our findings reveal that Topless proteins, key-regulators of growth-defense antagonisms, are a major target of the *U. maydis* effectome.

Long non-coding RNAs in the barley powdery mildew fungus

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The obligate biotrophic pathogens *Blumeria graminis* f.sp. *hordei* (*Bgh*) is one of devastating powdery mildews, *Bgh* can only infect *Hordeum vulgare* (barley) and causes huge yield losses. It is important to dissect the molecular interaction mechanism between *Bgh* and its host,

which can help develop strategies to protect barley. *Bgh* possesses relatively large genome (125 Mb) compared to the average ascomycete fungi (Frantzeskakis *et al.*, 2018). Coding genes in *Bgh* only represent a small part of genome: More than 75% is occupied by transposable elements (TEs) that exhibit low divergence, suggest a recent burst of TE in *Bgh*. Further, the repeat-induced polymorphism (RIP) that helps mutagenize TEs in fungi is absent. To regulate TEs, *Bgh* could employ DNA methylation, histone modification, and RNA interference (RNAi). We found long non-coding RNAs (lncRNAs) located on the antisense strand of TEs and hypothesize that lncRNAs play key functions in TE regulation. We performed stranded RNA sequencing of key stages of the asexual infection cycle at 0, 6, 18, 24, 72, and 120 hours past inoculation. We computationally and manually annotated 5,887 expressed lncRNAs. Locus-specific TE expression was quantified using the expectation-maximization (EM) method of Telescope to re-assign ambiguous reads (Bendall *et al.*, 2019). We identified 33 pairs of positively regulated *cis* (less than 10 kb distance) regulated (Pearson correlation > 0.7) and 14 pairs of negatively regulated ($P < -0.7$) lncRNA and mRNA. Further, we found 20 hub lncRNAs by weighted correlation network analysis (WGCNA). Our future research will focus on demonstrating physical interactions between lncRNAs and target molecules to unravel the mechanisms of lncRNA-controlled regulation in the obligate biotrophic plant pathogen *Bgh*.

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DNA hypomethylation accompanies defense priming and systemic acquired resistance

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Systemic acquired resistance is an inducible, broad-spectrum plant immune response that is associated with priming for enhanced defense. Priming involves epigentic modification of chromatin, such as covalent alteration to histones and DNA. Together, these chromatin changes seem to provide a long-lasting, epigenetic immune memory enabling very robust activation of defense genes upon future pathogen attack. In contrast to histone modification, little is known about the role of DNA (hypo)methylation in defense priming and systemic acquired resistance. However, it was shown that Arabidopsis mutants with reduced DNA methylation were generally more resistant to pathogens whereas Arabidopsis mutants with repressed expression of DNA demethylase genes were more susceptible to disease. We report

the genome-wide hypomethylation of DNA in primed systemic leaves of Arabidopsis plants with systemic acquired resistance. In addition, we show that two Arabidopsis T-DNA insertion mutants, that is *decreased dna methylation 1* and the DNA methyltransferases triple mutant *domains of rearranged methyltransferase 1/2* - *chromomethylase 3*, have reduced systemic acquired resistance and impaired inducibility of priming-responsive genes. This work serves as a starting point to shed more light into the contribution of DNA hypomethylation to the epigenetic immune memory of plants.

Abstracts der Vorträge: Session 3

Which factors influence establishment of Powdery mildew *(Erysiphe betae)* in sugar beet?

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INTRODUCTION

Powdery mildew of beet *Erysiphe betae* (Vanha) Weltzien is present in all parts of the world where sugar beet (*Beta vulgaris* L.) is cultivated. After introduction of sugar beet cultivation in new growing regions, infections with *E. betae* soon emerge, probably due to wind dispersal of conidia of other regions (Joa *et al.*, 2017). Its ubiquitous appearance characterizes the fungi as a generalist. However, disease progress can become fast and yield losses dramatically high, depending on cultivation region (El-Sheikh Aly *et al.*, 2020). In other cases, *E. betae* remains relatively easy to control (Vasel *et al.*, 2013). There are epidemiological reports of required temperature and humidity of the fungus (Drandarevski, 1969; Wolf, 2002; Kontradowitz, 2009). Our aim was to supplement the existing findings in order to define rates of germination, infection propability and disease severity under different climate regimes. These epidemiological data will be used to predict progress of *E. betae* infections in the field based on current weather data.

METHODS AND MATERIAL

Germination of conidia of *E. betae* has been examined under eight constant temperature regimes within the range of 0 to 35 °C during 24 hours in 2 h-intervals. The influence of the leaf surface on germination rate has been simulated by the use of cellulose film (32.5 g per m²) on agar plates. Infected plants with different infestation periods have been used as a source of conidia in order to assess the influence of the age of conidia on germination. In addition, influence of light (5,000 - 8,000 Lux) on germination has been determined at four temperature levels (12, 15, 20 and 27 °C) after 24 hours. Infection severity has been studied on young sugar beet plants (5 weeks old) at different temperatures (10 to 25 °C) and at different relative humidity levels (55 %, 70 – 90 %, >90 %) under a 16 hours light / 8 hours dark regime. The sugar beet plants were evenly inoculated to dripping point with the aid of Preval-sprayers using a suspension of 6.5 – 8 x 10^4 conidia per ml. Assessment was based on estimation of infection severity by the help of a magnifying glass ring light and was rated as percentage of infected leaf area. A stereo microscope (Zeiss SteREO Discovery.V20) was used to evaluate first occurance of mycelial growth and first sporulation on infected leaves at respective temperatures.

RESULTS

Germination rate was highly dependend on the age of conidia used for the testing. It decreased very fast when using conidia from plants infected more then two weeks ago. The use of cellulose mebrans enhanced germination rate significantly by 17 % on average compared to germination on pure water agar. Conidia obtained from two-week-old infestations on cellulose showed highest germination rates at temperatures between 25 and 30 °C. Illumination led to a significant increase in germination rate, especially at lower temperatures, and transferred the optimum temperature of germination to 20 °C. This was in accordance with infection trials on sugar beet plants, which showed highest disease severity at 20 °C after one and two weeks, respectively. The main difference between temperatures was the time until infections had established on the plants. After one week post inoculation, disease severity was 1.6 % at 10 °C and 36.9 % at 20 °C, respectively. Younger leaves (leaf no. 5 to 7) showed significantly higher disease severity then older leaves (leaf no. 3 and 4). Infection severity was highest under lowest relative humidity level (55 %). First mycelium growth was detected the second day after inoculation at 20 °C and 25 °C, respectively. Sporulation was first detected at 25 °C after 5 days post inoculation.

CONCLUSIONS

Assessment of germination has identified the vitality of conidia as an important factor of successful germination, which is the prerequisite for infection. Hence, fresh infections in the field bear a high risk of dissemination, whereas older infections may not be successful in futher spreading. Concerning temperature, there are hardly any limitations of germination during daytime. Likewise, infections are possible in a wide range of temperatures, although initial infection progress varies grately. Taking relative humidity data and different rates of initial growth and sporulation into account, there might be some potential for predicting the optimum timing of plant protection practice.

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Investigations on hot water treatment for the production of high-quality grapevine propagation material

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Grapevine trunk diseases (GTD) are one of the most destructive diseases of grapevine observed in many wine producing countries worldwide. GTD are caused by one or several xylem-inhabiting fungi including *Phaeomoniella chlamydospora* (Pch), *Phaeoacremonium minimum* (Pmi) and several members of the *Botryosphaeriaceae* (BOT) species. Endogenous pathogens can be disseminated through grafting of grapevines leading to significant losses in newly established vineyards and old vines. Hot water treatment (HWT) of dormant woody plant material is an environmentally safe and effective method in controlling GTD pathogens. Nonetheless, previous studies have shown contradicting results in the effectiveness of various HWT protocols against GTD pathogens. In addition, wounds are the main sites of infection by GTD pathogens and are susceptible to an infection for up to 4 weeks after pruning. The combination of using biological control agents (BCAs) such as *Trichoderma* species with other sanitation methods could be an effective long-term protection of pruning wounds in grapevine nurseries against GTD pathogens.

In this research project, the heat tolerance of the individual developmental stages of the pathogens (Pch, Pmi and BOT) is determined and the sensitivity of the individual developmental stages to HWT is assessed at different combinations of temperature and exposure time. Field experiments are carried out to observe the effect of HWT on the targeted fungal pathogens, where inoculated wood cuttings are treated in a hot water tank. The cuttings are grafted on rootstock, planted in nurseries and sampled for visual observations on plated samples. To determine the influence of a HWT on the growth of *Trichoderma*, a commercially available BCA product, Vintec®, *Trichoderma atroviride* strain SC1 (TASC1, Belchim Crop Protection Deutschland GmbH) is tested. Experiments are conducted with different temperature- time combinations at inoculated wood cuttings to determine the influence of a HWT on the growth of TASC1. The long term antagonistic effect of TASC1 following HWT is examined in a field study where scion cuttings are inoculated with GTD pathogens and treated with the combination of HWT and TASC1.

The results reveal valuable insights on the susceptibility of these pathogens to HWT at different temperature-time combinations and the impact of the treatment on the antagonistic potential of the BCA against GTD pathogens is evaluated.

Blowin' in the Wind: Wind Dispersal Ability of Phytopathogenic *Fusarium* in a Wind Tunnel Experiment

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Dispersal processes play an essential role in cereal diseases caused by phytopathogenic *Fusarium*. However, most empirical studies of *Fusarium* spore dispersal have focused on vertical transport by rain splash, while wind dispersal has been mostly neglected. In our research, which we would like to present, the focus was on determining *Fusarium* conidiospores' ability to disperse via wind under controlled conditions in a wind tunnel study (Hoffmann et al. 2021). Ten *Fusarium* species with diverse spore varieties were studied by placing them in the wind stream at wind velocities of 5 and 8 m s^{-1} and collecting them after 6 m and a period of 1 h using a newly developed air sampling box. Although spore concentrations were high in the releasing Petri Dishes, the tested isolates were recaptured in only 18 of 78 runs. *F. equiseti* and *F. cerealis* were the most frequently recovered species. Changing abiotic conditions, wind speed, and spore shapes had no significant effect on Fusarium spore recapture rates. For further studies, we have suggestions to include carrier media or mobile linkers combined with the wind dispersal of spores.

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FarmingIOS – hyperspectral imaging for non-invasive disease detection to improve efficiency of precision farming

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Despite recent advances in plant disease detection with optical sensor techniques which have proven functional in laboratory studies, these techniques are still not employed in agricultural practice due to measurement difficulties of hyperspectral imaging under field conditions and at a sufficient scale for practical applications.

The FarmingIOS project aims to integrate optical sensor-based pathogen detection into agriculture. Therefore, a combination of close-range hyperspectral imaging time series measurements of plant pathogen interactions and drone based multispectral measurements

has been applied. This approach allowed a detailed study of pathogen-specific changes to host plants and the optimization of their detection through the use of artificial intelligence on laboratory scale. The resulting detection methods were tested on field scale via UAV-based measurements with high throughput multispectral measurements, allowing a verification under field conditions and adaption of the symptom recognition algorithms.

Four selected crops (soybean, wheat, apple and grapewine) have been investigated during the project with the goal of automated disease map generation to precisely initiate plant protection measurements.

A UAS (Unmanned Aerial System) based monitoring system for plant diseases in field vegetable cultures

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For a successful production of field vegetable crops it is necessary to have the control over all relevant parameters in the field, such as moisture, nutrition, pests and diseases. As example, this information is needed to take countermeasures against appearing pests or diseases to avoid damages of the crop. The challenge is to recognize the pathogen infection as early as possible to minimize yield loss. This can be achieved through the consequent execution of field inspections to monitor the development of the crop from seeding to harvest. This monitoring is time consuming and expensive as staff with expertise in pests, diseases and physiological parameters is required. The DiWenkLa project establishes a monitoring system based on drones as carriers for multispectral and hyperspectral sensors. In connection to the field flights the image data is analyzed by different machine learning methods to achieve image data classification. At the end of the processing chain, the resulting disease map contains information on infestations and other heterogeneities including the information about where they are in the field. The GPScoordinates of stress hotspots can be exported from the image data to be used in precision agriculture to treat only the relevant areas instead of the whole field.

Cercospora leaf spot disease prediction and monitoring by an integrated approach based on optical sensor and environmental modeling in sugar beet

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Cercospora leaf spot (CLS) is one of the most damaging leaf diseases in sugar beet, and it can cause a yield reduction of up to 40%. Due to changing climate and environmental conditions,

CLS is more prevalent in warm areas and is spreading further in temperate regions. This increased disease pressure demands an early and accurate detection to avoid losses and evaluate the necessity of a management action according to integrated pest management. Recently, remote sensing techniques combined with machine learning techniques have proven their potential to detect and monitor CLS in sugar beet. However, to improve the accuracy and specificity of monitoring and detection, the integration of weather-based prognosis and remote sensing seems promising.

Therefore, various meteorological and environmental data were measured in experimental fields inoculated with Cercospora beticola. In parallel fields were overflown by drones mounted with multispectral cameras and scored by experts. The goal is to create a fused model that includes measurements from different domains for early detection of CLS infestations of fields. Therefore, the first step was to identify the most influential variables obtained from sensors and environmental data. Finally, the variables were included in a model according to their degree of importance and ease of measurement. As preliminary results, it was found that data from red and infrared bands of the camera, thermal sums of temperature, and cumulative sum of spore flight were most important for the detection of CLS.

After successfully integrating different data types, data from other sites and further measurement years are needed to confirm these obtained results.

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Engineered coumarin biosynthesis enhances crop resilience

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Coumarins have an important role in the defence of plants to biotic and abiotic stress. In addition, many coumarins have been attributed with human health-supporting properties. Such coumarins seem to be promising for sustainable, consumer-friendly crop protection. Feruloyl-6' hydroxylase 1 (F6'H1) is the key enzyme in the biosynthesis of the coumarin scopoletin. *F6'H1*-overexpressing plants accumulated scopoletin and its glycosidic storage form scopolin. Simultaneous overexpression of genes encoding MYB transcriptions factors, which boost the phenylpropanoid pathway, further enhanced coumarin levels in plants. To avoid possible detrimental effects of high levels of antioxidative coumarins in plants and to get the coumarins to the leaf surface where many pathogens would hit the plant, we chose

different strategies. Accumulation of different scopoletin derivatives was achieved by exploiting coumarin-biosynthesis genes of various plant species. Furthermore, we identified different transporters enabling enhanced secretion of coumarins to the leaf surface. Our future work will focus on controlling the activity of genes with a role in coumarin biosynthesis and secretion by regulated promoter activity. Overall, our strategy of tailored coumarin biosynthesis and secretion provides crops with enhanced capacity to fend off pathogens.

Abstracts: Poster Pitch Session 2

Identification of FRP peptides as novel phytocytokines

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Plants have to tightly regulate the magnitude of immune responses against potential threats to prevent excessive defense activation and ensure proper growth and development in the absence or ceasing of infection. One strategy employed by plants to control this growth-defense trade-off is a signaling cross-talk involving several classes of small endogenous peptides. In addition to their important function in diverse physiological processes, some peptides were shown to amplify or attenuate the strength of immune responses. In analogy to metazoan systems, these peptides can be referred to as phytocytokines. To identify novel phytocytokines, we mined publicly available gene expression data in *Arabidopsis thaliana* and found distinct members of a specific plant peptide family that exhibit a strong differential transcriptional regulation in response to flagellin 22 (flg22) treatment, suggesting a potential role in plant immunity. We found that a specific peptide gene of this family was transcriptionally down-regulated after flg22 treatment. Interestingly, overexpression of this *FLG22- REPRESSED PEPTIDE (FRP)* gene resulted in increased flg22-triggered responses and enhanced resistance to *Pseudomonas syringae pathovar tomato (Pto)*. To further investigate its role in immunity, we generated higher order CRISPR-Cas9 mutants eliminating *FRP* and five close homologous genes. The resulting mutants showed enhanced susceptibility to *Pto* infection, confirming a positive regulatory role of these peptides in antibacterial resistance. To further validate a role in immune responses, we tested synthetic peptides and observed that FRP treatment induced hallmark immune outputs and potentiated flg22-triggered responses. This suggests a function of mature FRP in pattern-triggered immunity. Moreover, we observed that a local application of FRP enhanced resistance against *Pto* in distal tissue, suggesting a role in systemic acquired resistance. Currently, we are focused on identifying receptors of these novel phytocytokines and to elucidate the mechanistic basis for FRP-mediated regulation of plant immunity.

Modulation of apoplastic papain-like cysteine proteases by root commensals of *Zea mays*

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Apoplastic papain-like cysteine proteases (PLCPs) are major hubs in plant immunity (1). PLCPs are common targets of pathogen effectors and were hypothesized to release microbe- and damage-associated molecular patterns. In maize leaves, we could show that PLCPs are activating salicylic acid (SA) related defenses. However, the role of PLCPs in the interaction with commensal bacteria is barely known. In maize roots, we found three apoplastic PLCPs being activated after salicylic acid treatment (2). We hypothesize that bacterial endophytes overcome plant immunity by inhibiting SA-associated root PLCPs. To test, if root-colonizing bacteria secrete proteinaceous inhibitor molecules, low- and high-molecular-weight fractions from culture supernatants of maize root-colonizing bacteria were analyzed. This screen identified PLCP-inhibitors in the high-molecular-weight fraction of *Pseudomonas putida*, as well as the low-molecular-weight fraction of *Stenotrophomonas maltophilia*. These results suggest that commensal, maize root-colonizing bacteria like *P. putida* and *S. maltophilia* inhibit host PLCPs. I will present our current progress on the identification of bacterial PLCP inhibitors. Understanding their role in root colonization and microbial community structure will advance our understanding about how commensal bacteria can engage with the plant while at the same time pathogens are restricted.

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Multiple mutagenesis of *Botrytis cinerea* by an improved CRISPR/Cas9 protocol reveals high redundancy of phytotoxic proteins for necrotrophic infection

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Botrytis cinerea is a necrotrophic plant pathogen characterized by a wide range of host plants. During invasion, it quickly kills the host cells and colonizes the dead tissue. Mechanisms that contribute to host killing during host invasion include secretion of CWDE, release of phytotoxic proteins and metabolites, tissue acidification and the activation of defence responses culminating in plant hypersensitive cell death. The precise role of the individual components during infection is not well understood. We have established CRISPR/Cas9 genome editing in *B. cinerea* (Leisen et al. 2020, PLoS Pathogens, 16, 1-32). The protocol was further improved by using a double sgRNA-RNP strategy in combination with transiently selected telomere

vector for repetitive marker-free gene deletions, which resulted in highly efficient generation of homokaryotic, marker free single or double mutants. By this means, we have constructed and characterized a series of up to 18-fold knockouts of genes encoding cell death inducing proteins (CDIPs) and two phytotoxic metabolites, botrydial and botcinin. Genome sequencing of a 12x mutant confirmed the deletions and revealed only few off-target mutations. The mutants showed generally decreased virulence with increasing numbers of deleted genes, but dependent on the infected host tissue (leaves of beans, tomatoes, maize, Arabidopsis; apple fruit), different effects of gene deletions were observed. Our data document one of the first systematic approaches to address functional redundancy of virulence factors of a pathogenic fungus, and the apparent absence of single major virulence proteins in *B. cinerea*.

Functional characterisation of rust effectors in *Ustilago maydis*

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Obligate biotrophic fungal pathogens, such as *Puccinia sorghi*, are amongst the emost devastating plant pathogens affecting agricultural important crops. For a successful colonisation of their host, plant pathogens secrete a set of effectors targeting molecules, pathways and structures of their host. Whilst many effectors of biotrophic, hemibiotrophic and necrotrophic pathogens have been identified and functionally characterised, studying effectors of obligate biotrophic pathogens remains challenging due to the lack of efficient genetic transformation and gene deletion techniques. In this study, we deploy the *Ustilago maydis* – maize pathosystem to characterise putative effectors from the common rust *Puccina sorghi*. To this end we identified 40 putative *U. maydis* effectors with homologues in *P. sorghi* and assessed their impact on *U. maydis* virulence. So far, eight of the *U. maydis* gene candidates have been identified as virulence factors. Currently, we are integrating the *P. sorghi* homologues in *U. maydis* to test if they can restore virulence in the heterologous system.

The dynamic regulation of transposons in the barley powdery mildew fungus

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The powdery mildew fungi are obligate biotrophic pathogens of wild and crop plants. The cereal powdery mildew *Blumeria graminis* infects grasses and cereals in a host-specific manner, e.g., the barley powdery mildew (*B. graminis* f.sp. *hordei*, *Bgh*) can only infect

Hordeum vulgare (barley). Transposable elements make up >75% of the genomes of cereal powdery mildews and can be the source of genetic variation and genome instability. We observed transcriptional induction of transposons during early infection, suggesting dynamic regulation of these elements. Since the fungal repeat-induced point mutation (RIP) mechanism is absent in *B. graminis*, we hypothesize that transposons are regulated by epigenetic means. We study whole-genome cysteine methylation, histone modification, and RNA interference in the barley powdery mildew. We discovered long spliced antisense RNAs at loci of transposon replication genes, which may regulate transposons by RNA interference. Cysteine methylation levels appear to be high in certain transposable element classes. We further detected the histone K9 acetylation but not K27 methylation in conidia. Our work unravels how dynamic transposon regulation contributes to virulence in an obligate biotrophic fungal plant pathogen.

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Transcriptional regulation of effector genes in smut fungi

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Smut fungi are biotrophic plant pathogens which cause severe diseases in the aerial parts of major crops, including maize, wheat, barley and sugarcane. Secreted fungal proteins, called effectors, antagonize plant defenses to facilitate the biotrophic development and virulence activity of the fungus. Recent studies using comparative genomics have identified a number of novel secreted effectors, which can be grouped into core effectors, which are conserved and essential in processes related to infection, as well as accessory effectors, which are thought to play more specific roles in virulence (Thines, 2019). Although, a number of possible *Ustilago maydis* transcriptional regulators has been previously proposed (Lanver *et al.,* 2017), little is known about the evolution of transcriptional regulation in smut effector genes. Our work focuses on the study of effector gene promoters in smuts in an attempt to elucidate the key cis-regulatory elements essential for the transcriptional regulation of those genes. A

combination of transcriptomic and genomic pipelines and tools was used with the aim to inravel sequence motifs which could be associated with spatial and/or temporal expression of *Ustilago maydis* effectors. Additionally, comparative studies between *Ustilago maydis* and *Sporisorium reilianum* effector promoters has shed light into a putative promoter element, here named CAC-motif, which has been further studied for its functionality as a transcriptional regulatory element.

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Tailoring coumarin biosynthesis in plants

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The hyperaccumulation of secondary metabolites with antimicrobial activity is promising for sustainable crop protection. We showed that overexpression of a gene encoding feruloyl-CoA-6'-hydoxylase-1 (F6'H1) causes the accumulation of the coumarin scopoletin and its glycoside scopolin in transgenic plants including Arabidopsis and soybean. Accumulation of the two coumarins is associated with enhanced defense and resistance to disease. To avoid possible detrimental effects of high scopoletin levels on plant growth and development, we secured its conversion to a more antimicrobial but less deleterious derivative by co-expressing genes encoding various coumarin-biosynthetic enzymes. To facilitate the controlled co-expression of these genes, we took advantage of the viral 2A peptide technology. The 2A peptide allows for the expression of combined transgenes and subsequent separate translation of the encoded proteins by ribosomal skipping. We confirmed that by using the 2A peptide the expression of two separate plant biosynthetic enzymes from one coding sequence was possible. A high 9:1 ratio of separate to fused proteins was confirmed by immunodetection. The separate enzymes were both functional thus catalyzing the conversion of scopoletin into one of its derivatives in planta. Furthermore, using the 2A peptide allowed expression driven by the same promoter, which ensured uniform regulation of expression and prevented promoter silencing in transgenic plants. The use of 2A-peptides when combining transgenes in plants not only presents new opportunities to fine-tune coumarin biosynthesis, but also provides a valuable tool of genetic engineering for sustainable crop protection.

Soil-born endophytic fungi antagonize plant-parasitic root-knot nematodes in tomato

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Plant-parasitic nematodes, especially sedentary root-knot nematodes (*Meloidogyne* spp.), are a serious threat to many crop plants causing worldwide tremendous economic losses. Due to the ban of the most chemical nematicides, there is a strong need for new environmentally friendly alternatives. One of such promising options is the utilization of endophytic fungi, such as different species of Sebacinales family. There are already auspicious results from various studies showing considerable positive impact of those fungi on plant growth, yield and tolerance/resistance against different biotic and abiotic stresses [1, 2]. Our previous *in vitro* studies indicate that for instance *Serendipita indica* acts against cyst nematodes in a model plant Arabidopsis [3]. Therefore, the objective of this work was more applied investigation of the possible application of two Sebacinales fungi, *S. indica* and its domestic relative *Serendipita williamsii*, against the root-knot nematodes in tomato (*Solanum lycopersicum* L.). For that, we inoculated tomatoes with *M. incognita* (*MIG*) and both Sebacinales species *in vitro* and in pots. For both conditions, we demonstrated the significant increase in growth parameters in all plants colonized with both fungi irrespective of nematode infection in comparison to control plants and plants infected only with *MIG*. Further, we observed similar significant reduction in number of galls induced by *M. incognita* in plants colonized with both endophytes. These results clearly show that the fungal colonization counteracts the negative impact of nematode infestation in tomato. An additional analysis of the expression of defense and hormone-related marker genes at 3 and 7 days after inoculation showed significant differences between variants indicating expressional reprogramming of the host in this tripartite interaction. Our data demonstrate the beneficial effects of both *Serendipita* species on plant growth as well as their antagonistic properties against *MIG*. These findings can be used for the further development of new environmentally harmless nematicides based on these endophytes or on fungus-derived substances.

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The Role of the Ustilago maydis GATA transcription factor Nit2 for the successful infection of maize and nitrogen utilization during biotrophy

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The biotrophic phytopathogenic fungus Ustilago maydis makes use of a variety of molecular tools to successfully infect its host plant maize (Zea mays). Pathogenic development of U. maydis is initiated by the fusion of two compatible haploid sporidia, resulting in the formation of a dikaryotic hypha followed by the formation of appressoria to achieve the invasion of the plant cell by biotrophic hyphae. Both, the transition from saprophytic, yeast-like cells to biotrophic hyphae, as well as the modulation of host metabolism and suppression of plant immune defenses, requires extensive transcriptional regulation.

In previous studies, the transcription factor Nit2 was shown to play a major role for the utilization of non-favored nitrogen sources like minor amino acids or nucleobases in sporidia. Nit2 transcript amounts were increased during biotrophy compared to axenic culture and deletion of Nit2 resulted in retarded filamentation and reduced virulence in planta, indicating that favored nitrogen sources may be limiting during biotrophy. Employing ΔNit2 mutants, we identified 33 potential Nit2 targets in planta, which largely differed from the known Nit2 targets in sporidia. We currently analyze the role of Nit2 and selected targets for nitrogen utilization in planta by steady state metabolite and 15N stable isotope flux analysis, as well as by fluorescent reporters. Since we also found that Nit2 targets are overrepresented among b-regulated genes, we aim at resolving the role of Nit2 in the transcriptional network during early pathogenic development.

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Cell-type specificity in the biotrophic interaction of *Ustilago maydis* and its host plant maize

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Ustilago maydis is a member of smut fungi which can infect all aerial parts of *Zea mays*. This group of plant pathogens is characterized by a narrow host range and the well-studied *U. maydis* provides an important model for host-pathogen interactions to understand the biology and function of effectors and biotrophic fungal lifestyle. Cell-type-specific transcriptome profiling of *U. maydis* during tumor formation revealed a set of specifically and strongly upregulated effector genes during hypertrophy (hypertrophic mesophyll tumor-HTT) which causes enlargement of mesophyll cells upon infection (1). Among ten HTT specific effector genes, two of them (Hte1and Hte2) were found to be required for full virulence in two maize lines, Golden Bantam (GB) and Early Golden Bantam (EGB), respectively. In addition, a deletion mutant of Hte3, a paralogue of Hte2 identified in subsequent mass spectrometry (MS) analysis, showed significant reduction in tumor formation in EGB.

To understand, how the Hte-effectors act on the molecular level, we performed immunoprecipitations (IP) followed by MS from extracts of infected maize leaves using HA-tagged versions of the effectors. Surprisingly we obtained not only putative plant targets, but also found that the effectors Hte1, Hte2 and Hte3 interact with each other inside the plant tissue.

To investigate a putative complex being formed by the cell-type specific effectors, Co-immunoprecipitation (Co-IP) and split-luciferase complementation experiments were performed. In addition, both double- and triple deletion mutants for the Hte-effectors were tested for virulence in both GB and EGB maize lines. Strikingly, neither double- nor triple deletion mutants exhibited additive effects but showed a similar reduction of virulence to that of single deletion mutants in both GB and EGB maize lines, which provides additional evidence for their functional independence in *U. maydis* pathogenicity.

This functional characterization of the individual and cooperative activities of secreted effector proteins sheds new light on the molecular basis of *U. maydis*-induced tumor formation.

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Abstracts der Vorträge: Session 4

Functional analyses of *Serendipita indica* effector candidates in redirecting phytohormone signalling and activating beneficial effects in *Arabidopsis*

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The fitness of land plants is highly dependent on beneficial symbioses that are based on tight molecular communication between all partners [1]. Because of this, microbes evolved strategies based on the secretion of specific proteins (termed effectors). These effectors were first described to suppress plant immunity but later found to target and alter a plethora of plant signalling pathways [2]. The beneficial fungus *Serendipita indica* enhances biotic and abiotic stress resilience as well as growth promotion in hosts. These beneficial effects depend at least partially on the ability of the fungus to redirect hormone signalling [3].

In order to understand to what extent effectors participate in hormone rewiring, we studied 106 *in silico* identified *S. indica* effector candidates (SIEC). Co-IP and yeast-two-hybrid screens detected a complex interaction network between SIECs and Arabidopsis proteins. Comparative interactomics further detected differences and overlapping targeting of *S. indica* and plant pathogen effectors with increased interactions of SIECs with hormone pathways. Consistent with this, protoplast-based functional analyses revealed that about half of the identified SIEC are able to modulate hormone signalling pathways in hosts. Those hormone functions were further confirmed in whole plant assays. Specific SIEC overexpression was changing root growth, which corresponded to signalling functions demonstrated in protoplast assays.

Taken together, by combining network interactomics and functional protoplast assays we were able to identify previously unknown hormone functions of Arabidopsis proteins targeted by SIECs. We further validated to what extent SIEC-based modulation of phytohormone pathways confer beneficial effects.

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Characterization of cellular pathways involved in the RACB-mediated susceptibility of barley towards the powdery mildew fungus *Blumeria graminis* f.sp. *hordei*

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Plant disease susceptibility can arise from a range of different factors. Pathogens secrete effectors to subdue or bypass plant defenses, but plant proteins can also be involved in facilitating infection. These plant proteins, so-called susceptibility factors, are often essential for full establishment of the pathogen and plant development. Barley RACB, a small monomeric G-protein, has been shown to be such a susceptibility factor. RACB has been extensively studied in the interaction with the powdery mildew fungus *Blumeria graminis* f. sp. h*ordei* (*Bgh*), which might target RACB directly to successfully infect plant cells. However, the molecular mechanisms behind RACB-mediated susceptibility remain poorly understood. Using transgenic RACB overexpression plants and co-immunoprecipitation followed by MS-analysis, we identified new potential interaction partners and cellular pathways involved in RACB-dependent disease susceptibility. Among these, phospholipid-binding proteins of plant and fungal origin are heavily overrepresented. Hence, we aim to identify the role of these proteins in the barley-*Bgh* interaction and provide insight into how phospholipids can influence fungal accommodation in barley epidermal cells.

*Mo*Pl1 and *Mo*Nudix are required for full virulence of *Magnaporthe oryzae*

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The hemibiotrophic fungus *Magnaporthe oryzae* is the causal agent of rice blast disease. Because the pathogen infects also other sweet grasses such as barley, millet and wheat, which account for a large proportion of the world's staple food supply, the fungus is a threat for global food security.

To infect plants, the pathogen secretes proteins, referred to as effectors, to manipulate the host plant immune response. Aiming to understand this process at the molecular level, we characterized the function of *MoPL1* and *MoNUDIX*, two genes which encode for a pectate lyase and a Nudix-hydrolase, respectively.

Gene deletion mutants of *MoPL1* revealed its requirement for full virulence of the pathogen. Interestingly, also a constitutive expression led to a diminished virulence most probably due to liberation of danger associated molecular patterns. Nudix-hydrolases are known to cleave a wide range of nucleoside diphosphates linked organic substrates. Transcription peaked at 48-72 hours after inoculation, pointing to a potential function in the switch from a biotrophic to a necrotrophic life-style. Using CRISPR/Cas9 technology and marker-free *in locus* complementation, we could confirm that deletion of both copies of the gene led to a loss in virulence on barley. Gene function of the orthophosphate releasing enzyme was investigated by computer assisted modeling, analysis of mutants constitutively expressing a mRFP-tagged version of the protein and *in vitro* assays. A putative nucleic acid processing function of the protein is likely because a release of free inorganic phosphate from dATP and genomic DNA was observed.

Cross-kingdom RNA transfer in barley and powdery mildew interaction

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Powdery mildews are biotrophic fungal pathogens. The interactions between *Blumeria graminis f.sp. hordei* and *Hordeum vulgare* is agronomically important. sRNA species of ca. 20-25 nucleotides in length play a key role in gene silencing via RNA interference (RNAi). During RNAi, endogenous microRNAs (miRNAs) or short interfering RNAs (siRNAs) inhibit gene expression by either conditioning homology-dependent degradation of messenger RNAs (mRNAs) or by preventing mRNA translation. Recent advances suggest that small RNAs might play a major role in cross-kingdom RNA interference. In this work, we dissected the infected leaves into separate fractions representing different compartments that are key for understanding the pathogenic system. These were the epidermis, mycelium, haustoria, a fraction of microsomes, and extracellular vesicles isolated from the intercellular spaces (apoplast) of infected or non-infected leaves. We found that sRNA isolated from different compartments were very different, this is significant because it indicates that there were selective processes for generation and possibly transport of the sRNA interaction. We further

identified *bona fide* microRNA and micro RNA like RNAs (milRNAs), the differential expression, and the processes they regulate.

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Interplay of MLO and exocyst complex proteins in localized secretion

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Though it is known for decades that MLO loss-of-function mutants confer durable resistance against powdery mildew fungi in a variety of plant species, the genuine function of MLO proteins remains elusive. Some studies, however, found that certain *Arabidopsis thaliana mlo* mutants show phenotypes linked to polar secretion including absent root thigmomorphogenesis, pollen tube overgrowth or reduced fertility. In this context, we found that a deviation of callose deposition in the powdery mildew resistant *Atmlo2 Atmlo6 Atmlo12* triple mutant resembles a phenotype observed in trichomes deprived of the exocyst complex subunit *At*EXO70H4. Resting upon this congruity between *mlo* and *exo70H4* mutants, we speculated that MLO proteins may be involved in the secretory pathway. Taking advantage of a procedure for the separataion of leaf tissue and trichomes, we analyzed the cell wall of wild-type and mutant trichomes by various means including histochemichal dyes, the biochemical quantification of monosaccharides and Fourier-transform infrared spectroscopy. Furthermore, we used luciferase complementation and yeast two-hybrid to test different EXO70 proteins for a direct interaction with clade V *AtMLO* proteins. Our data indicate that deviations of the cell wall in *mlo* and *exo70H4* trichomes are beyond a simple defect in callose deposition. In addition, the outcomes of protein-protein interaction experiments imply a physical interaction of certain EXO70 proteins with MLO2, MLO6 or MLO12 probably mediated by the MLO C-terminus.

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Defining the septin interactome and its function in appressorium-mediated plant infection by the rice blast fungus Magnaporthe oryzae

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In 2021, it was estimated that billion humans lacked healthy nutrition and ensuring food security is therefore a significant global challenge. Rice is a staple food for more than 50% of the world population and as the rice blast fungus destroys enough each year rice to feed more then 60 million people, its control could make a vital contribution to feed the world sustainably. Blast disease is initiated by formation of a specialized infection cell, called an appressorium. Turgor generation within the appressorium of up to 8MPa, enables the fungus to develop a rigid penetration peg to breach the rice cuticle. *Magnaporthe oryzae* possesses six septin GTPases, which play major roles in appressorium-mediated infection. Septins form an heterooligomeric ring at the appressorium pore, which requires the four core septins Sep3, Sep4, Sep5 and Sep6. Two additional non-core septins, Sep7 and Sep8 are responsible in *M. oryzae* for formation of a range of membrane and cytoskeleton-associated structures. To determine the role of septins during appressorium-mediated plant-infection, we have carried out high throughput yeast two hybrid assays, coupled with *in vivo* immunoprecipitation mass spectrometry (IP-MS) experiments to define the septin interactome. For each septin we have identified a wide range of interaction partners during appressorium development, including polarity determinants, cytoskeletal components, and a range of regulatory proteins. Interestingly we observed that Sep7 interacts with Sep3, Sep4, Sep5 and Sep6 specifically during early appressorium formation, 4h after conidial germination, forming a plasma membrane-associated complex. Sep8, which contains a transmembrane helix, also interacts with each septin and may link septins to the plasma membrane. When considered together, we provide evidence that septins form a key regulatory hub that orchestrates re-modelling of the cytoskeleton, cell membrane and cell wall, enabling symmetry breakage and re-polarisation at the base of the infection cell. This also provides new information for potential disease-control strategies to be devised.