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Gunther Döhlemann (Hrsg.)

59. Jahrestagung des DPG-Arbeitskreises Mykologie und 43. Jahrestagung des DPG-Arbeitskreises Wirt-Parasit-Beziehungen 2024 an der Universität Bonn

Zusammenfassungen der Arbeitskreisbeiträge

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Deutsche Phytomedizinische Gesellschaft e.V.

59. Jahrestagung des DPG-Arbeitskreises

Mykologie

43. Jahrestagung des DPG-Arbeitskreises

Wirt-Parasit-Beziehungen

07./08. März 2024

an der

Universität Bonn

Zusammenfassungen der Arbeitskreisbeiträge

Bericht und Kurzfassungen der DPG Arbeitskreis Tagungen Mykologie und Wirt-Parasit-Beziehungen, 7./8. März 2024 an der Universität Bonn

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Jahrestagung der DPG-Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“ am 07./08. März 2024 an der Universität Bonn

Liebe Kolleginnen und Kollegen,

in diesem Dokument finden Sie das Programm der Beiträge zur gemeinsamen Tagung der DPG-Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“ am 07./08. März 2024.

Um alle Beiträge innerhalb von zwei Halbtagen berücksichtigen zu können, haben wir sowohl für den Donnerstagnachmittag als auch den Freitagvormittag zwischen den 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 in die Poster Pitch Sessions verschoben haben.

Das Zeitfenster für einen Vortrag beträgt 20 min (15 min Redezeit, 5 min Diskussion). Wir bitten Sie, die Zeit einzuhalten und die Themen der Vorträge gut einzuführen, damit auch Nichtfachleute davon profitieren. Da sich die Poster Pitch Sessions in den vergangenen AK-Treffen bewährt haben, wollen wir diese noch junge Tradition auch diesmal weiterführen. Dabei stellen die AutorInnen ihre Poster zunächst in Form einer **maximal 2-minütigen Kurzpräsentation** vor. Wir bitten hierzu um **Vorab-Zusendung einer aus 1-2 Folien bestehenden Zusammenfassung der Highlights Ihres Posters als pdf-Datei bis spätestens zum 4. März.**

Wir freuen uns auf das Treffen in Bonn und auf einen lebhaften wissenschaftlichen Austausch.

Mit den besten Grüßen
Gunther Döhlemann und Marco Thines

PROGRAMM

Donnerstag, 07. März 2024

13:00 Uhr BEGRÜSSUNG

VORTRAGS-SESSION 1 (Leitung: Ulrich Schaffrath)

13:10 Uhr Nick Dunken (Uni Köln): A nucleoside signal generated by a fungal endophyte regulates host cell death and promotes root colonization

13:30 Uhr Laura Rehneke (JLU Gießen): Analysis of *Serendipita indica* effector function in *Arabidopsis thaliana* signalling pathways and confer of benefits

13:50 Uhr Wei Shi (Uni Köln): Identification of AVRA effector targets in barley

14:10 Uhr Yoon Joo Lee (Uni Köln): The Pec effector complex of *Ustilago maydis* interferes with carbohydrate metabolism in maize

14:30 Uhr Alex Wegner (RWTH Aachen): A small Magnaporthe effector hijacks plants' phosphate starvation signaling

14:55 bis 15:15 Uhr POSTER PITCH SESSION 1 (2 min short presentations)

1-1: Merle Bilstein-Schloemer (Uni Köln): Functions of powdery mildew avirulence effectors inside barley host cells

1-2: Mariem Bradai (TU München): Unraveling the molecular mechanism of a potential downstream interactor in RACB signaling in barley disease susceptibility

1-3: Tomás Alberto Cortés Román (Uni Bonn): Transposon mutagenesis in the biotrophic fungus *Ustilago maydis*

1-4: Ruben Eichfeld (Uni Köln): Time-resolved transcriptomics reveal a mechanism of host niche defense: beneficial root endophytes deploy a host-protective antimicrobial GH18-CBM5 chitinase

1-5: Ida Faust (RPTU Kaiserslautern-Landau): Unveiling the Veil: Candidate 15 from *Botrytis cinerea*

1-6: Janina Werner (Uni Köln): A recombinant hybrid provides insights in the regulation of effector genes contributing to tumor formation of *Ustilago maydis*

1-7: Ena Šečić (Uni Gießen): Transcriptome – wide analyses of sRNAs in the interaction between *Arabidopsis thaliana* and *Serendipita indica* – how to find interesting targets?

1-8: Sophia Hein (TU München): Model experiments on the effects of the Fusarium mycotoxin Deoxynivalenol in barley

1-9: Mamoona Khan (Uni Bonn): Tips- (TOPLESS interacting proteins) effectors from *Ustilago maydis*

1-10: Henriette Leicher (TU München): Endogenous FER-RALF signaling is involved in successful host colonization of powdery mildew on *Arabidopsis thaliana*

1-11: Xinyi Liu (TU München): The contribution of transposable elements of *Blumeria hordei* to cross kingdom compatibility with its barley host

15:15 bis 16:00 Kaffeepause & Postersession 1

VORTRAGS-SESSION 2 (Leitung: Gunther Döhlemann)

16:00 Uhr Sabrine Nasfi (JLU Gießen): Functional validation of small RNAs and cross-kingdom communication in the mutualistic interaction of *Arabidopsis thaliana* and *Serendipita indica*

16:20 Uhr Tobias Müller (RPTU Kaiserslautern-Landau): Under Pressure: Unravelling the Penetration Power of *Botrytis cinerea*

16:40 Uhr Andrea Sistenich (RWTH Aachen): Marker and readout genes for defense priming in *Pseudomonas cannabina* pv. *alisalensis* interaction aid understanding systemic immunity in Arabidopsis

17:00 Uhr Severin Einspanier (CAU Kiel): High-resolution disease phenotyping reveals distinct resistance strategies of tomato crop wild relatives against necrotrophic pathogens

17:20 Uhr Christina Steidele (TU München): Network inference reveals barley transcription factors and their predicted targets in Fusarium head blight

Ab 18:30 Uhr Gemeinsames Abendessen

Freitag, 08. März 2024

VORTRAGS-SESSION 3 (Leitung: Marco Thines)

08:40 Uhr Antônia Finkler (Uni Göttingen): Identification of Cercospora spp. as part of the late cycle disease complex of soybean in Brazil

09:00 Uhr Jan Nechwatal (LfL Freising): *Phytophthora cinnamomi* detected in an outdoor Vaccinium stand in Southern Germany

09:20 Uhr Johanna Hoffmann (Uni Hohenheim): A new Biofungicide for the Control of Major Cereal Diseases

09:40 Uhr Sabine Kind (JKI Dossenheim): Application of mycorrhiza to overcome apple replant disease growth depression

10:00 Uhr Remco Stam (CAU Kiel): Presence-absence variation of and recombination of NLR genes in crop wild relatives

10:20 bis 10:45 Uhr POSTER PITCH SESSION 2 (2min short presentations)

2-1: Behnoush Hosseini (Uni Hohenheim): Characterization of *Diaporthe* spp. infecting soybean in Germany

2-2: Fluturë Novakazi (Uni Rostock): Influence of temperature and climatic origin on growth behaviour of *Pyrenophora teres f. teres*

2-3: Andrea Tobian Herreno (CAU Kiel): Population diversity of wheat pathogen *Zymoseptoria tritici*: A field study in the UK

2-4: Marion C. Müller (TU München): Avirulence depletion assay: combining bulk segregant analysis with artificial selection to identify novel virulence determinants in *Blumeria graminis*

2-5: Jakub Rzemieniewski (TU München): CEP signalling coordinates plant immunity with nitrogen status

2-6: Weiliang Zuo (Uni Köln): Functional adaptation of Sts2 effector orthogroup in smut fungi

2-7: Zarah Sorger (Uni Köln) Exploring the Role of GH 25 in Fungal Lifestyle and Microbial Antagonism

2-8: Johanna Stehle (CAU Kiel): Identification and characterization of miR398GGT of *Gaeumannomyces graminis* var*. tritici* and its possible role in plant-fungus interactions

2-9 Nassim Safari (RPTU Kaiserslautern-Landau): Addressing redundant roles of phytotoxic proteins for necrotrophic infection of *Botrytis cinerea* by multi-k.o. mutagenesis

2-10: Ruchi Tiwari (Uni Köln): Molecular intricacies of smut-induced host resistance toward secondary infection

2-11: Sakshi Sharma (RPTU Kaiserslautern-Landau) Searching powdery mildew-resistant oaks for Palatine Forest rejuvenation under climate change

10:45 bis 11:30 Uhr Kaffeepause & Postersession 2

VORTRAGS-SESSION 4 (Leitung: Monika Heupel)

11:30 Uhr Julian Maroschek (TU München): Fusarium elicitor sensing by MIK2 RLKs is conserved among different plant families

11:50 Uhr Christian Müller (Uni Jena): The histone 3 lysine 36 methyltransferase Ash1 represses effectors during saprotrophic growth in both S*. reilianum* and *U. maydis* but affects virulence and morphology only in *S. reilianum*

12:10 Uhr Alejandra Vielba-Fernandez (RPTU Kaiserslautern-Landau): Revealing the role of Reactive Oxygen Species (ROS) in the pathogenesis of *Botrytis cinerea*

12:30 Uhr Hendrik Seide (CAU Kiel): Investigating molecular mechanisms underlying the quantitative resistance against *Sclerotinia sclerotiorum* in oilseed rape (*Brassica napus*) using genetic and functional genomic approaches

12:50 Uhr Sebastian Schade (TU München): CrRLK1L-RALF signaling controls powdery mildew susceptibility

13:20 Uhr Verabschiedung

Abstracts oral presentations

A nucleoside signal generated by a fungal endophyte regulates host cell death and promotes root colonization

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The intracellular colonization of plant roots by the beneficial fungal endophyte *Serendipita indica* follows a biphasic strategy. After an early biotrophic phase, the interaction transitions to a host cell death phase restricted to the epidermal and cortex layers of the root. Host cell death contributes to the successful accommodation of the fungus during the beneficial interaction in the roots of Arabidopsis thaliana. How host cell death is initiated and controlled is largely unknown. Here we show that two fungal enzymes, the ecto-5'-nucleotidase SiE5NT and the nuclease SiNucA, act synergistically in the apoplast at the onset of cell death to produce deoxyadenosine (dAdo), a potent cell death inducer in animal systems. The uptake of extracellular dAdo, but not the structurally related adenosine (Ado), activates a previously undescribed cell death mechanism in A. thaliana. Mutation of the equilibrative nucleoside transporter ENT3 in A. thaliana results in resistance to cell death triggered by extracellular dAdo and reduced fungal-mediated cell death during root colonization. A library screen of A. thaliana T-DNA insertion lines identified a toll/interleukin-1 receptor nucleotide-binding leucine-rich repeat (TIR-NLR) protein as an additional intracellular component in dAdo-triggered cell death. Mutation of this previously uncharacterised TIR-NLR, which we have named ISI (induced by S. indica), affects host cell death, fungal colonization and growth promotion, suggesting a key role in the regulation of root cell death and plant-microbe interaction. Our data show that the combined activity of two fungal apoplastic enzymes leads to the production of a metabolite that, upon uptake, triggers TIR-NLR-modulated plant cell death, providing a link to immunometabolism in plants.

Analysis of *Serendipita indica* effector function in *Arabidopsis thaliana* signalling pathways and confer of benefits

Rehneke L[1]; Osborne R[2,3]; Lehmann S[2,4]; Roberts J[2]; Altmann M[5]; Altmann S[5]; Zhang Y[6]; Köpff E[7]; Dominguez-Ferreras A[2]; Okechukwu E[2]; Sergaki C[2]; Rich-Griffin C[2]; Ntoukakis V[2]; Eichmann R[1]; Shan W[6]; Falter-Braun P[5,8]; Schäfer P[1]

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Plant colonisation of land is highly depended on close relationships with symbionts to withstand environmental challenges [1]. Microbial strategies to interact with and colonise plants include the secretion of small proteins, termed effectors. Effector function was first described in host immunity suppression by pathogens, but they were also identified during beneficial plant-symbiont interactions [2]. The beneficial fungus Serendipita indica modulates host hormone signalling to improve host fitness and colonised plants show increased growth, biotic and abiotic stress resistance [3]. While S. indica utilizes effectors that alter hormone signalling during colonisation [4], it is still unclear how symbiont effectors are involved and contribute to the activation of beneficial effects. In a recent publication we described 106 newly identified S. indica effector candidates (SIECs). Comparative interactome analyses between SIECs and pathogen effectors identified exclusive symbiont targets. Additionally, SIECs specifically alter hormone signalling and can be used as tools to uncover complex plant signalling networks. Expression of single SIECs in Arabidopsis increased seedling growth [5]. Moreover, SIECs target stress-related host proteins and modulate biotic and abiotic stress signalling. SIEC expression increased abiotic stress resistance and tolerance to pathogens in plants.

We aim to comprehensively analyse specific SIECs and target function in complex hormone and stress signalling pathways. Based on our study we are able to identify molecular mechanisms of symbiont-host interaction and signalling modulations that confer plant environmental stress resilience.

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Identification of AVR_A effector targets in barley

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Blumeria hordei (*Bh*) causes the powdery mildew disease of barley. In resistant barley lines, the RNase-like *Bh* AVR_A effectors are recognized by immune receptors encoded at the barley *Mildew locus a* (*Ml*a). The maintenance of AVR_A effectors in the *Bh* population despite their detection by MLA receptors suggests that the effector's intrinsic function is crucial for *Bh* virulence. However, these virulence functions are currently entirely unknown.

To identify the AVR_A virulence function we aim to identify their targets inside barley host cells. For this, we applied a combination of *in vivo* proximity-dependent protein labelling and co-immunoprecipitation using AVR_A-expressing stable transgenic barley lines. We identified multiple candidate interactors of AVR_{A1}, AVR_{A7}, AVR_{A9}, AVR_{A10} and AVR_{A13} and are now following up on the individual candidate interactors using one-to-one interaction assays. Our data suggests that AVR_{A13} but no the other AVR_A effectors targets a member of the barley Strubbelig Receptor Family (SRF) to modulate host immunity. AVR_{A13} can interact with the cytoplasmic domains of only certain barley SRF family member isoforms. Together our initial data suggests that a) the intrinsic functions of individual *Bh* AVR_A effectors differ despite the fact that the effectors are structurally related and b) that members of the SRF may modulate immunity towards powdery mildew and potentially other pathogens.

The Pec effector complex of *Ustilago maydis* interferes with carbohydrate metabolism in maize

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Ustilago maydis, a biotrophic smut fungus which secretes a cocktail of effectors in a spatiotemporally regulated manner to trigger tumor formation in maize. It manipulates the host's metabolism, redirecting starch and glucose accumulation towards developing tumors, thereby turning them into strong sinks. Comprehensive cell-type-specific transcriptome profiling of *U. maydis* during tumorigenesis revealed a set of highly upregulated effectors involved in hypertrophy, leading to enlargement of mesophyll cells upon infection [1]. Here, we show that the effectors Pec (Primary metabolite Effector Complex) 1-3 are hypertrophy-associated effectors, being essential for the full virulence of *U. maydis*. Co-immunoprecipitation coupled with mass-spectrometry analysis of maize extracts infected with *U. maydis* revealed intra-host interactions of Pec1-3, suggesting a potential effector complex. Moreover, we observed a reduction and mislocalization of starch in tumors of plants infected with *U. maydis* Pec single-, double-, and triple knockout mutants as compared to uninfected cells, indicating that Pec effectors act cooperatively to disrupt carbon metabolism in maize. To gain insight into the potential cooperative mechanism of Pec effectors within the host, we performed a large-scale mass spectrometry analysis which identified Pec1 as the central Pec-effector. In particular, Pec1 interacts with maize SnRK1 (SNF1–related protein kinase 1), a central integrator of the plant stress and nutrient homeostasis activated by phosphorylation under energy starvation. Quantitative phosphoproteomics analysis of Pec1 knockout mutants compared to wild-type infected plants revealed that SnRK1, TPS (trehalose-6-phosphate synthase), and other glucose-stimulating enzymes are highly phosphorylated in response to wild-type infected plants. These results suggest that Pec1 disrupts the antagonistic relationship between SnRK1 and T6P (trehalose-6-phosphate) via TPS, leading to metabolic reprogramming that reduces sugar uptake but increases extracellular sugars in *U. maydis* -induced tumors.

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A small *Magnaporthe* effector hijacks plants' phosphate starvation signaling

Wegner A[1], McCombe CL[2], Zamora CS[3], Casanova F[1], Aditya S[2], Greenwood JR[2], Wirtz L[1], de Paula S[3], England E[2], Shang S[2], Ericsson DJ[4], Oliveira-Garcia E[3], Williams SJ[2], Schaffrath U[1]

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The phytopathogenic ascomycete *Magnaporthe oryzae* is best-known as the causal agent of the rice blast disease. Besides rice, *M. oryzae* also infects other economically important sweet grasses such as barley, millet and wheat. To enable host cell colonization, the fungus deploys an arsenal of effectors, which manipulate the host plant immune system. We delved into the fungal-plant interactome by spotlighting a small effector with a Nudix hydrolase domain. Utilizing CRISPR/Cas9 mediated approaches, we investigated the impact of the effector-encoding gene *MoNUDIX*, present twice in the genome of *M. oryzae*. *MoNUDIX* double-deletion mutants exhibited significantly reduced virulence, manifested by much smaller lesions on leaves compared to the fungal wild type isolate. Live cell imaging with mRFP-tagged *MoNUDIX* confirmed secretion of the effector via the biotrophic interfacial complex into the host cell cytoplasm. We identified inositol pyrophosphates as the effectors' substrate. The concentration of inositol pyrophosphates is sensed by plants to monitor their phosphate status and depletion results in induction of the phosphate starvation response which, as a side effect, dampens the plants immune response. Here, we unveiled the pathogen's strategy of secreting *Mo*NUDIX into host cells, which mimics phosphate starvation, leading to a suppression of immunity and finally promotion of virulence.

Functional validation of small RNAs and cross-kingdom communication in the mutualistic interaction of *Arabidopsis thaliana* and *Serendipita indica*

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Cross-kingdom RNA interference (RNAi) describes the bidirectional exchange of small (s)RNAs, between microbes and their hosts. These sRNAs regulate gene function in the respective interacting organism, which requires the molecular interaction of sRNAs with ARGONAUTEs (AGOs) enzymes. We use *Arabidopsis thaliana* (*At*) and the endophytic fungus *Serendipa indica* (*Si*) as a model system to uncover the role of fungal sRNAs (*Si*sRNAs) in a mutualistic symbiosis. Using *ago* mutants, we confirmed the dependency of the symbiosis on specific Arabidopsis AGOs. AGO immunoprecipitation assays (AGO-IPs) using *At* root samples colonized with *Si* were performed to identify *Si*sRNA potentially involved in the *Si*-*At* interaction. Together with an sRNAomics approach encompassing mRNA-Seq, degradome-Seq, and sRNA-Seq datasets from *At* roots colonized with *Si* (see the abstract of Šečić *et al.*), we aim at identifying all *Si*sRNAs (*Si*sRNA atlas) that are involved in the establishment of the *Si* symbiosis. To quantify post-transcriptional gene silencing of host target genes, *Si*sRNA candidates are transiently and stably expressed in *At* protoplast and whole plants, respectively. We then analyse the expression of *Si*sRNAs by stem-loop PCR and determine the cleavage sites of their corresponding target genes by 5' RLM-RACE. The studies will reveal to which extent *Si*sRNAs facilitate plant colonisation and whether they participate in the regulation of plant growth responses and development.

Under Pressure: Unravelling the Penetration Power of *Botrytis cinerea*

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The devastating pathogen Botrytis cinerea infects a broad spectrum of host plants, causing great damage. During invasion, Botrytis rapidly kills plant cells, nourishing their cell walls and subsequently feeds on their contents. To this end, it secretes a cocktail of cell wall degrading enzymes, phytotoxic proteins and metabolites. Additionally, many fungi produce specialized appressoria as invasion structures that generate high turgor-driven invasive pressures to force their way into plants. However, for necrotrophs, including Botrytis, the biomechanics of penetration and its contribution to virulence are poorly understood. Here we use a combination of quantitative micromechanical imaging and CRISPR-Cas guided mutagenesis to show that Botrytis uses substantial invasive pressure, in combination with strong surface adherence, but without requirement of phytotoxic proteins, for penetration. We found that the fungus establishes a unique mechanical geometry of the penetrating hypha that develops over time during invasion. This process is dependent on the actin cytoskeleton. Interference of force generation by blocking actin polymerization was found to decrease Botrytis virulence, indicating that also for necrotrophs with less specialized invasion structures, mechanical pressure is important in host colonization. Our results demonstrate for the first time mechanistically how a necrotrophic fungus such as Botrytis employs this “sophisticated force” approach, in addition to the secretion of lytic proteins and phytotoxic metabolites, to overcome plant host resistance.

Marker and readout genes for defense priming in *Pseudomonas cannabina* pv. alisalensis interaction aid understanding systemic immunity in Arabidopsis

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Following localized infection, the entire plant foliage becomes primed for enhanced defense. However, specific genes induced during defense priming (priming-marker genes) and those showing increased expression in defense-primed plants upon rechallenge (priming-readout genes) remain largely unknown. In our A*rabidopsis thaliana* study, genes *AT1G76960* (function unknown), *CAX3* (encoding a vacuolar Ca2+/H+ antiporter), and *CRK4* (encoding a cysteine-rich receptor-like protein kinase) were strongly expressed during *Pseudomonas cannabina* pv. alisalensis-induced defense priming, uniquely marking the primed state for enhanced defense. Conversely, *PR1* (encoding a pathogenesis-related protein), *RLP23* and *RLP41* (both encoding receptor-like proteins) were similarly activated in defense-primed plants before and after rechallenge, suggesting they are additional marker genes for defense priming. In contrast, *CASPL4D1* (encoding Casparian strip domain-like protein 4D1), *FRK1* (encoding flg22-induced receptor-like kinase), and *AT3G28510* (encoding a P loop-containing nucleoside triphosphate hydrolases superfamily protein) showed minimal activation in uninfected, defense-primed, or rechallenged plants, but intensified in defense-primed plants after rechallenge. Notably, mutation in only priming-readout gene *NHL25* (encoding NDR1/HIN1-like protein 25) impaired both defense priming and systemic acquired resistance, highlighting its previously undiscovered pivotal role in systemic plant immunity.

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Sistenich et al. (2024) Marker and readout genes for defense priming in *Pseudomonas cannabina* pv. alisalensis interaction aid understanding systemic immunity in Arabidopsis. Scientific reports, DOI: 10.1038/s41598-024-53982-5

High-resolution disease phenotyping reveals distinct resistance strategies of tomato crop wild relatives against necrotrophic pathogens.

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Quantitative disease resistance (QDR) is commonly considered as an incomplete, yet broad-spectrum form of resistance controlled by a complex interplay of multiple minor-effect genes. The regulatory factors controlling QDR have not yet been adequately described and functional characterization has been impeded because of the limitation of accurate tools for measuring QDR-phenotypes at high resolution and throughput. We optimized a high-throughput phenotyping platform to characterize QDR-phenotypes of wild tomato species against the necrotrophic pathogens *Alternaria alternata, Alternaria solani* and *Sclerotinia sclerotiorum.* Accordingly, we were able to characterize patterns of QDR-diversity at the species- and accession-level among wild tomatoes plants. Here, we show that wild tomatoes possess of an astonishing degree of inter- and intraspecific QDR-diversity. Furthermore, we observe the existence of distinct QDR-strategies. Each QDR-strategy, like the retardation of disease outbreak or deceleration of lesion growth, might be controlled by discrete functional cues translating to different QDR-phenotypes, like lag-phase duration or lesion growth rate. We further investigated potential regulatory mechanisms underlying different QDR strategies, gaining initial insights into the contribution of transcriptional reprogramming to quantitative resistance. Our results help understand whether QDR-phenotypes are conserved in a broad panel of wild tomatoes and can be used for more targeted breeding purposes aiming for durable resistance.

Network inference reveals barley transcription factors and their predicted targets in Fusarium head blight

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Fusarium head blight (FHB) can be caused by various Fusarium spp. and poses a significant threat to barley production. So far, no major FHB resistance gene was identified, and our current knowledge indicates the existence of quantitative resistance in barley against FHB. Quantitative FHB resistance likely involves multiple genes with a complex interplay. To gain a deeper understanding of such interplay, we analyze transcriptomic data to identify co-expressed genes and assess their contribution to resistance or susceptibility. We utilized weighted gene correlation network analyses for identifying co-expressed genes that simultaneously associate measured phenotypes. Employing gene regulatory networks helped to identify potential key regulatory transcription factors and their associated targets for future functional validation. This integrated approach aims at a comprehensive understanding of the molecular mechanisms underlying quantitative resistance to FHB in barley.

Identification of Cercospora spp. as part of the late cycle disease complex of soybean in Brazil

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Diseases of soybean caused by Cercospora spp. are endemic throughout the world's soybean production regions and are part of the late cycle disease complex. The genus Cercospora has historically been underrepresented in terms of species diversity due to a dependence of differentiation on morphological characteristics, symptomology, and host associations (Groenewald et al., 2013). Based on this, two species (C. kikuchii and C. sojina) were initially known to infect soybeans. However, recent research, including molecular analytical methods, shows that numerous species can be associated with the disease. Therefore, the objective of this study was to identify the predominant species of Cercospora associated with typical symptoms on soybean in Brazil and investigate the pathogenicity of the identified species in greenhouse experiments. For this purpose, 43 isolates of Cercospora spp. from Brazil were analyzed using a multi-locus phylogenetic approach with five different loci from the gDNA. Furthermore, cloning was performed for three loci on 11 leaf samples, to correctly identify the species present. Of the 43 isolate samples, 41 were identified as Cercospora sp. Q, one as C. kikuchii and one as C. cf. sigesbeckiae. In the cloned leaf samples, C. sp. Q was the predominant species in all 11 samples analyzed. However, C. kikuchii, C. cf. flagellaris, C. cf. nicotianae and C. sp. P were also detected in almost all samples, although at much lower frequencies. Furthermore, infection of soybean plants under greenhouse conditions was achieved for the predominant species (C. sp. Q). This study was able to confirm that Cercospora sp. Q is currently the most frequent species of Cercospora infecting soybean in Brazil and that the late cycle disease complex can involve more than one species of Cercospora simultaneously. However, further studies need to be conducted to identify improved markers for the differentiation between Cercospora species.

Phytophthora cinnamomi detected in an outdoor Vaccinium stand in Southern Germany

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Phytophthora cinnamomi together with P. cactorum was detected in soil samples from an outdoor northern highbush blueberry (Vaccinium corymbosum) stand in Bavaria, southern Germany. Both pathogens have not been reported from highbush blueberry in Germany before, and both were equally virulent to this host in inoculation assays. Phytophthora cactorum is a widely distributed species in nurseries and (semi)-natural habitats like forests, parks and gardens. In contrast, reports of outdoor occurrences of P. cinnamomi in Germany are rare due to its well-known sensitivity to frost. Our findings once more indicate P. cinnamomi's potential to survive Central European winter periods that due to climate change tend to be increasingly mild. Young commercial V. corymbosum nursery plants purchased during this study were also infested with P. cinnamomi, indicating a high level of contamination in commercial blueberry production. The risk of the spread of invasive exotic plant pathogens through the use of infested nursery plants is also clearly demonstrated.

A new Biofungicide for the Control of Major Cereal Diseases

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Zymoseptoria tritici, Blumeria graminis and *Ramularia collo-cygni* are among the most important pathogens of cereals in Europe. They can cause high crop yield and quality losses without suitable control, which often involves synthetic chemical fungicides. These are not authorized for organic farming and harbour the risk of resistance development by pathogens, which has become an increasing problem over the last decades. With the Green Deal, the EU has set the target of reducing the use of synthetic chemical fungicides by 50 % by 2030. Natural alternatives that can effectively combat these plant diseases are therefore sought after to keep yields stable and to secure a sufficient food supply. BAS 824 F, a biological fungicide based on sulfur and a second naturally occurring compound, currently under development at BASF SE, was tested for its effectiveness against *Z. tritici, B. graminis* and *R. collo-cygni* in comparison to commercial multi-site and single-site fungicides. The effects on spore germination and fungal growth were investigated in in vitro tests as well as in field trials. The results showed an inhibitory effect of BAS 824 F on the spore germination of *Z. tritici* and *B. graminis*. In field trials with all three pathogens, BAS 824 F performed equally well or better than the commercial multi-site fungicides. Single-site fungicides outperformed all other fungicides, but the effect tended to be improved by additional application of BAS 824 F. Overall, the results support the further development of BAS 824 F as an effective biological fungicide for practical use in plant protection.

Application of mycorrhiza to overcome apple replant disease growth depression

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Apple Replant Disease (ARD) is a phenomenon occurring after repeated planting of apple plants at the same site. Plants show growth depressions and fruit yield decline. It is known that biotic factors contribute to ARD but overall it is not well understood. To date the most effective way to control the disease is by the application of broad-spectrum chemical fumigants. The project ORDIAmur aims to develop strategies to overcome ARD by more environmentally friendly methods. The project includes the application of mycorrhiza and the effect on the microbial community in the apple roots. In the scope of greenhouse trial a qPCR method was successfully applied to detect the mycorrhization by *Rhizophagus irregularis* of the apple roots depending on different soil treatments. Tagetes pre-planting facilitated R. irregularis abundance in the apple roots. A correlation between the abundance of R. irregularis and plant growth was not detectable.

Presence-absence variation of and recombination of NLR genes in crop wild relatives

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The NLR (Nucleotide-binding domain and Leucine-rich Repeat) gene family plays a pivotal role in pathogen resistance. Both intra and interspecifically NLR show large diversity. What fuels NLR diversity within a species, and especially the role of recombination and copy-number variation herein, have remained elusive. Here we present the results of two different studies. The first study looks at the role of copy number variation, especially absence/presence variation (PAV) in over 200 accessions of the wild tomato species Solanum chilense. We build a rigorous pipeline to validate the identification of PAV of NLRs using target capture sequencing, then show that PAV is larger within populations than between populations and lower than in other species. PAV appears to be limited to certain NLR clusters, and using a redundancy analysis; we find limited evidence of PAV being linked to environmental gradients, suggesting that maintenance of NLR diversity is linked to population dynamics and intraspecific diversity of NLR is required for species survival, but highly species and gene-specific. In the second study, we utilized a unique dataset from Solanum Americanum to investigate the contribution of recombination to NLR diversity. We employed an approach involving truncation and MCL clustering of 18 PacBio target capture datasets and unequivocally demonstrated that recombination plays a role in fueling NLR diversity within Solanum americanum. Notably, this phenomenon is not uniform across all NLRs but appears to selectively affect specific subfamilies, including the recently identified Rpi-amr1 clade. This selective impact on sensor NLRs provides a novel perspective on the evolutionary dynamics governing plant-pathogen interactions. Overall we conclude that PAV and recombination combined can be driving forces for NLR diversity within a species and that analyses of these processes can potentially guide identification of new functional resistance alleles.

Fusarium elicitor sensing by MIK2 RLKs is conserved among different plant families

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The histone 3 lysine 36 methyltransferase Ash1 represses effectors during saprotrophic growth in both *S. reilianum* and *U. maydis* but affects virulence and morphology only in *S. reilianum*

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To successfully colonize maize plants the two smut fungi *S. reilianum* and *U. maydis* must adapt and alter their gene expression profile during saprotrophic growth to an expression profile suited for their biotrophic growth stage. To successfully establish and maintain the biotrophic growth of *S. reilianum* and *U. maydis*, effector genes are timely expressed, however, the mechanisms controlling effector gene expression are not fully understood. We show through RNA-Seq analysis that the histone 3 lysine 36 methyltransferase Ash1 is responsible for repressing expression of at least 50% of all predictable effector genes during saprotrophic growth in both *S. reilianum* and *U. maydis*. Furthermore, deletion of Ash1 in *S. reilianum* led to a morphological change from lemon-shaped cells to filamentously growing cells, but deletion of Ash1 in *U. maydis* did not change morphology. Also, virulence was only reduced in the *S. reilianum* Δash1 deletion strains but not in *U. maydis* Δash1 deletion strains. We complemented the *S. reilianum* Δash1 deletion strains with the Ash1 coding sequences of the four smut fungi, *S. reilianum*, *S. scitamineum*, *U. hordei* and *U. maydis*. We found that the lemon-shaped morphology and effector repression was only restored when the deletion strain was complemented with Ash1 of either *S. reilianum* or *S. scitamineum*. These results indicate that Ash1 in *S. reilianum* and *U. maydis* may have a common function, repressing effector genes, and an *S. reilianum*-specific function, controlling morphology and virulence. Elucidating the mechanism of Ash1 function will help to better understand the plant infection process of these related maize pathogens.

Revealing the role of Reactive Oxygen Species (ROS) in the pathogenesis of *Botrytis cinerea*

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The rapid release of production of Reactive Oxygen Species (ROS), the so-called oxidative burst, constitutes the first line of defense of plant cells against pathogenic attacks. Due to their highly reactive nature, superoxide ions and hydrogen peroxide can cause direct damage to pathogens. However, they also act as signaling molecules for the activation of other defense mechanisms, such as callose deposition and the activation of defense-related genes. In certain cases, ROS-induced internal signaling may result in hypersensitivity response (HR) and programmed cell death. While this is a powerful resistance response against biotrophic pathogens, necrotrophs such a *Botrytis cinerea* are believed to trigger the oxidative burst and to induce HR as an infection strategy. In this case, pathogens need to overcome all HR-associated defence mechanisms. It is therefore believed that necrotrophic fungi require effective ROS detoxification mechanisms. However, data regarding the roles of ROS for infection of *Botrytis* are controversial. Therefore, our studies attempt at clarifying the roles of the oxidative burst in the plant-*Botrytis* pathogenic interaction.

By luminescence-based measurements of H_2O_2 with Arabidopsis leaf discs treated with various ROS-manipulating chemicals, secreted toxic proteins of Botrytis, and infected with *Botrytis* wild type and knock-out mutants lacking multiple secreted phytotoxic proteins, the oxidative burst was quantified. Additionally, the principal role of the NADPH oxidase isoform RbohD during the *Botrytis* infection, the main source of extracellular ROS generation during plant defense, has been confirmed using an *rbohD* mutant of *Arabidopsis*. To deepen the understanding of the role of RbohD on *Botrytis* attack, inoculations were conducted using different stages of fungal spore germination. Intruigingly, the RbohD-mediated oxidative burst was found to be inhibitory for infection by ungerminated spores, but not for pre-germinated spores used as inoculum. To investigate the role of ROS detoxification by *Botrytis* during infection, mutants were generated using CRISPR-Cas technology, targeting five genes previously identified as being involved in ROS detoxification. Preliminary experiments indicate that they don't play a major role for infection. Furthermore, the relationship between *Botrytis*, HR and defence gene expression was analysed. These experiments, along with planned future research,

aim to clarify the dynamics and roles of extra- and intracellular ROS production in the interaction between *Botrytis* cinerea and its host plants.

Investigating molecular mechanisms underlying the quantitative resistance against *Sclerotinia sclerotiorum* in oilseed rape (Brassica napus) using genetic and functional genomic approaches

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Sclerotina sclerotiorum is the causal agent of Sclerotinia stem rot (SSR) and has a vast host range spanning over 400 species, including oilseed rape (*Brassica napus*). Effective control of SSR is difficult as resistance resources within the gene pool of *B. napus* are limited and the use of fungicides is increasingly restricted. Recently, we have identified new QTLs for SSR resistance within the genome of *B. villosa*, a wild Brassica species, and successfully integrated them into oilseed rape. Here, we report the identification of QTLs for SSR resistance in oilseed rape, the underlying genes and mechanisms by using genetic and functional genomic approaches. Our results will provide new insights into the genomic structure of the QTLs for SSR resistance and the molecular interactions between oilseed rape and *S. sclerotiorum*.

CrRLK1L-RALF signaling controls powdery mildew susceptibility

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Abstracts poster pitches

1-1 Functions of powdery mildew avirulence effectors inside barley host cells

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Upon infection of barley, the biotrophic powdery mildew fungus *Blumeria hordei* (*Bh*) secretes a variety of effector proteins to the host. The effector's intrinsic function is to promote fungal virulence (virulence function). In resistant barley lines, some of these effectors are recognised by the barley *Mildew locus A* immune receptors (MLA receptors). The recognition of effectors by immune receptors is associated with host cell death and designated as the effector's avirulence (avr) function. The *Bh* effectors recognised by MLA receptors are called AVR_A effectors. Although the establishment of plant immunity through the recognition of AVR_A effectors presents a major disadvantage for the fungus, a multitude of AVR_A effectors are maintained in the fungal genome, suggesting an essential function of the effectors in the development of virulence, fungal proliferation, and mildew disease.

We investigate the molecular mechanisms underlying the virulence function of *Bh* AVR_A effectors and determine whether avirulence function (activation of the MLA receptors) is connected to virulence function. We are in particular interested in *AVR_{a13}* as the majority of *Bh* isolates carry this avirulent effector gene, suggesting that AVR_{A13} virulence function is non-redundant. We determine AVR_{A13} avirulence function by the initiation of MLA13-mediated cell death upon co-expression of the respective genes in *Nicotiana benthamiana* leaves. To measure AVR_{A13} virulence activity, we have established to assay AVR_{A13}-mediated inhibition of *Arabidopsis thaliana* immunity towards *Pseudomonas syringae* pv. tomato. This read out allows us to elucidate whether the AVR_{A13} virulence and avirulence function are linked or exerted independently from each other. For this, we mutated and exchanged residues in AVR_{A13} to identify mutant proteins which have lost the ability to activate MLA13. We then tested whether loss of avirulence function is connected to the loss of interaction with a newly identified putative AVR_{A13} host target. Our initial data suggests that escaping MLA13 recognition through diversification of AVR_{A13} is directly associated with the loss of host target interaction. This data may explain why loss of recognised *AVR_{a13}* is rare in the *Bh* population.

1-2 Unraveling the molecular mechanism of a potential downstream interactor in RACB signaling in barley disease susceptibility

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Rho GTPases are master regulators of cellular signalling processes in eukaryotes. In plants Rho-like ROPs/RACs are involved in polarization, hormonal signaling, and plant immunity or susceptibility to diseases. In barley (*Hordeum vulgare L.*), we found that RACB is required for full susceptibility towards Blumeria hordei infection and supports the accommodation of fungal infection structures (1,2,3). Furthermore, RACB´s physiological function was found in cell polarity, in particular nucleus positioning, polar growth of epidermal root hair cells and asymmetric cell division in leaf epidermal cells (2, 3). These data support that the susceptibility function of RACB probably lies in its role in cell polarity, which may be co-opted by the pathogen for invasive ingrowth of its haustorium (3). Nevertheless, how RACB supports fungal penetration success and which other host proteins coordinate this process is incompletely understood. During recent years, we found that this is achieved by RACB-downstream scaffold protein that bridge RACB to cytoskeleton and membrane organization during fungal invasion and haustorium expansion (3). To date, among the canonical ROP interactors in barley, ROP Interactive CRIB-domain (RIC), and ROP Interactive Partner (RIP), have been characterized to interact with RACB (4,5). Mechanistically, RICs and RIPs were suggested to be scaffolding proteins that mediate binding between RACB and so far unknown downstream proteins. Recently, among the obtained putative interaction partners of RIPb, we identified a Calcium-dependent lipid-binding protein, that could provide a link between the function of RACB in the accommodation of fungal haustoria in barley and calcium signalling.

1-3 Transposon mutagenesis in the biotrophic fungus *Ustilago maydis*

TAC Román

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1-4 Time-resolved transcriptomics reveal a mechanism of host niche defense: beneficial root endophytes deploy a host-protective antimicrobial GH18-CBM5 chitinase

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Associations between plants and beneficial root-endophytic fungi enhance plant performance by improving nutrient uptake, abiotic stress tolerance and disease resistance. To successfully colonize different host plants and defend their host niche against competing microbes, but also to cooperate with beneficial bacterial members of the microbiota, root endophytes such as Sebacinales secrete a multitude of tightly regulated effector-proteins and carbohydrate-active enzymes. However, the functions, specificity, and regulation of these proteins remain poorly understood. In this study, we employ time-resolved transcriptomics to analyse the gene expression profiles of two Sebacinales members interacting with organisms from different kingdoms of life. We identified crucial genes for plant colonization and intermicrobial competition, including a fungal GH18-CBM5 chitinase specifically upregulated in response to the phytopathogenic fungus *Bipolaris sorokiniana*. This chitinase protects the plant hosts against the pathogen, reducing fungal biomass and disease symptoms in barley and Arabidopsis thaliana. Our findings shed light on interaction partner specific gene expression in Sebacinales endophytes, with potential applications in enhancing plant health and resilience.

1-5 Unveiling the Veil: Candidate 15 from *Botrytis cinerea*

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Botrytis cinerea, causal agent of the grey mold, secretes numerous proteins, toxic metabolites and enzymes to infect host plants. During a recent secretome screening several unknown cell-death inducing proteins (CDIPs) have been identified (Jeblick et al. 2023). However, numerous identified potential CDIPs remain uncharacterized. Among all candidates, Bcin14g03970 (Candidate 15) displayed toxic activity in transient expression. Here we start unveiling the function of Candidate 15 (potential glucanosyltransferase) by combining transient gene expression, recombinant expression in *E. coli* and confocal laser-scanning microscopy. So far, our results point towards secretion and toxic activity of Candidate 15 in *Nicotiana benthamiana* but the underlying molecular mechanism needs to be investigated.

1-6 A recombinant hybrid provides insights in the regulation of effector genes contributing to tumor formation of *Ustilago maydis*

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Smut fungi infect economically important crops including barley, sorghum, wheat and maize. The majority of the smuts infect their host systemically and replace the inflorescences by teliospores, i.e. *Sporisorium reilianum f. zeae* infecting maize systemically to cause head smut disease. In contrast, the model organism *Ustilago maydis* forms distinct tumors locally at sites of infection on both maize leaves and inflorescences. *U. maydis* and *S. reilianum* are closely related, have similar genomes in size and synteny and infect the same host, *Zea mays*, providing a promising basis for interspecific hybridization.

To investigate, how the different effector gene sets of *U. maydis* and *S. reilianum* contribute to the different infection styles, a recombinant hybrid was generated. The hybrid successfully colonizes maize and reveals a *S. reilianum*-like phenotype without the formation of tumors and teliospores.

We used RNAseq to get insights in genome compatibility and gene expression levels in the binuclear hybrid strain. We discovered 219 differentially expressed 1:1 effector orthologs in the hybrid with distinct gene expression patterns. One of the identified patterns shows a downregulation of the *U. maydis* effector ortholog in the hybrid compared to the wild type. We hypothesized that *U. maydis* effector genes that are downregulated in the non-tumor forming hybrid might play a role in tumor formation. To test this hypothesis, we performed infection assays with knock-out mutants of the respective effector genes, which identified two novel virulence factors with a role in tumor formation.

In a next step, we overexpressed transcription factors (TFs) which are activated during host infection by *U. maydis.* Strikingly, overexpression of one conserved TF between the two species triggered the hybrid strain to induce tumor formation. The timing as well as the level of expression of the conserved TF is crucial for tumor formation. Finally, we aim to identify the effector orthologs regulated by this TF to unravel and reconstruct the molecular basis of *U. maydis* induced tumorigenesis.

1-7 Transcriptome – wide analyses of sRNAs in the interaction between *Arabidopsis thaliana* and *Serendipita indica* – how to find interesting targets?

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Small (s)RNAs are short non-coding RNAs, with regulatory roles in development and stress-response. As major factors in gene downregulation via RNA interference (RNAi), sRNAs silence mRNAs while attached to Argonaute (AGO) proteins. Canonically, sRNAs with known endogenous functions are classified in well-conserved micro (mi)RNA families. In turn, sRNAs exchanged between organisms during host-microbe interactions, termed cross-kingdom (ck)RNAs, modulate gene expression in the respective interacting partner. We explain how to analyze transcriptome-wide RNA datasets from *Arabidopsis thaliana* (*At*) roots colonized with the beneficial root endophyte *Serendipita indica* (*Si*). Based on genomic origin of sRNAs and targets, an identity (e.g. miRNA and/or ckRNA) can be assigned. The induction level during an interaction, relative to controls, is crucial for ranking of relevance. Association with RNA-binding proteins (e.g. AGO) solidifies the functional designation. Lastly, expression of target mRNA is an indication of the sRNA's phenotypic footprint. In our analyses, mRNA-Seq, degradome-Seq and sRNA-Seq were used to classify sRNAs and find their targets. Together with AGO-IP analyses (Nasfi *et al.*), our aim is to discover and validate sRNAs regulating symbiotic benefits in host plants, such as growth promotion and tolerance to stresses. A suitable functional validation pipeline studying *Si*sRNAs and their role in establishing mutualism is explored (please see abstract of Nasfi *et al).*

1-8 Model experiments on the effects of the Fusarium mycotoxin Deoxynivalenol in barley

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1-9 Tips- (TOPLESS interactiong proteins) effectors from *Ustilago maydis*

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1-10 Endogenous FER-RALF signaling is involved in successful host colonization of powdery mildew on Arabidopsis thaliana

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1-11 The contribution of transposable elements of *Blumeria hordei* to cross kingdom compatibility with its barley host

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2-1 Characterization of Diaporthe spp. infecting soybean in Germany

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Species of the large and complex genus Diaporthe can infect many different crop species and in several instances cause severe diseases. Systematics in the genus are complex because the species are morphologically very similar and the features that can be used to distinguish different species can also be overlapping so that it is almost impossible to distinguish the species based on morphological criteria. In addition, most of the species can infect several different host plants and any given host plant can be infected by different Diaporthe spp.. In the past this led to considerable confusion since mycologists have based species definitions in the genus Diaporthe on hosts that are infected. The correction of the Diaporthe phylogeny based on DNA sequence data is still ongoing. In the course of surveying Diaporthe spp. associated with soybean in central Europe we first found the four established soybean pathogens D. caulivora, D. eres, D. longicolla, and D. novem on soybean seeds, further described them and established quadruplex qPCR diagnostics for these species. Very recently we found and isolated three additional Diaporthe species from field samples in Baden-Württemberg, namely D. foeniculina, D. rudis, and D. goulteri. While D. foeniculina and D. rudis are established soybean pathogens and have already been observed in southern Europe, D. goulteri was first isolated from sunflower in Australia and later from genista plants in Italy. Apparently we are the first to find it on soybean. We are now in the process of characterizing our new isolates. At this year's DPG mycology meeting, we will present phylogenies including isolates of all seven Diaporthe species found in Germany. We will also show figures describing the morphologies of the three species found more recently and provide the first descriptions of D. goulteri on soybean.

2-2 Influence of temperature and climatic origin on growth behaviour of *Pyrenophora teres* f. *teres*

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To understand how fungal plant pathogens might respond to climate change, it is necessary to collect data on their growth behaviour under contrasting conditions and their current thermal adaption. We conducted a common garden experiment with five Pyrenophora teres f. teres isolates originating from five diverse locations. Ten replicates per isolates were tested for their growth rate under 12°C, 16°C, 20°C and 26°C. Isolates originating from regions with the lowest fluctuation in annual temperature showed higher growth rates at 16°C and 20°C than at 26°C. The isolate from the region with the highest annual temperature showed the slowest overall growth rate. However, the growth rates of the two isolates from the regions with the lowest and highest fluctuation of mean annual temperature, did not differ significantly from each other, but were significantly lower compared to the other three isolates. A clear and significant GxE interaction was observed, as 12°C and 26°C influenced the growth of the isolates in the same way, i.e. with higher annual temperature fluctuations, growth rates increased. No indication for thermal adaptation for the studied isolates could be found, implying the pathogen is not genetically limited under climate change. However, the number of isolates and their origin was quite limited, hence further studies are needed to allow more conclusive results.

2-3 Population diversity of wheat pathogen *Zymoseptoria tritici*: A field study in the UK

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Zymoseptoria tritici is a highly diverse wheat pathogen that has developed mutations for fungicide resistance in multiple genes and presents a challenge for traditional farming methods. We used a population genomics approach to assess the number of populations present in two fields in the UK and also understand the underlying structure of the diversity found. We show that there is only one population with a maximum genetic distance of 0.0044 compared to the average distance of 0.0016. No population structure exists in the assessed fields. Still, there is a high genetic diversity where the isolates obtained from the same lesion or plant can be genetically distant among the population regardless of geographical distance in the scale assessed.

2-4 Avirulence depletion assay: combining bulk segregant analysis with artificial selection to identify novel virulence determinants in *Blumeria graminis*

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2-5 CEP signalling coordinates plant immunity with nitrogen status

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2-6 Functional adaptation of Sts2 effector orthogroup in smut fungi

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Ustilago maydis is a fungal pathogen that causes common smut disease in maize. On maize seedlings, *U. maydis* induces the formation of two types of tumor cells, which are derived from the cell re-division of bundle sheath and the enlargement of mesophyll, respectively.

In our previous study, we conducted a cross-species transcriptome analysis to compare the regulation of one-to-one effector orthologs between *U. maydis* and its close relative, *Sporisorium reilianum*, which causes head smut on maize. We have identified an effector orthogroup that is differentially regulated in the respective pathogens during seedling infection[1]. The ortholog from *S. reilianum* was unable to restore the reduced virulence in the *U. maydis* mutant, indicating effector neo-functionalization during speciation[1]. Functional characterization of the *U. maydis* ortholog (UmSts2, small tumor on seedlings) revealed that UmSts2 acts as a novel fungal transcriptional activator, which transports into the host nucleus to activate the expression of maize leaf developmental regulators to regulate the hyperplasic tumor formation[2]. Interestingly, the SrSts2 ortholog is an active transcriptional activator, but does not induce the up-regulation of the same leaf developmental regulators[2]. Sts2 orthologs are identified only in a few smut fungi. How they adapt and contribute to the virulence of different pathogens remain unclear.

In this study, we experimentally tested the *trans*-activation activity of all Sts2 orthologs found in the sequenced smut species. In addition, an ancestral Sts2 protein was reconstructed to determine the function of Sts2 in effector adaptation. Using AlphaFold prediction we successfully identified two regions that are responsible for the 3D structure variation between UmSts2 and SrSts2. Swapping these regions in SrSts2 enabled it to rescue the reduced virulence of the *U. maydis* UmSts2 knockout mutant, suggesting their significant role in the neo-functionalization.

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2-7 Exploring the Role of GH 25 in Fungal Lifestyle and Microbial Antagonism

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Plants are colonized by a multitude of microbes, which interact not only with its host, but also display complex interaction networks within the microbial community. A scale-free high resolution network analysis of the leaf microbiome of *A. thaliana* revealed that most of these interactions are antagonistic [1]. The pathogenic oomycete *Albugo laibachii* identified as a hub microbe in shaping the microbial community. Moreover, basidiomycete yeast *Moesziomyces bullatus ex Albugo on Arabidobsis* (short: *MbA*) significantly decreases *A. laibachii* virulence (2). RNA sequencing revealed genes involved in the inhibition of *A. laibachii*. This approach identified *MbA* candidate genes encoding putative secreted proteins. Out of these four were putative glycoside hydrolases (GHs). The deletion of gene g2490 (GH25) resulted in an almost complete loss of *MbA* antagonistic activity against *A. laibachii* [2].

This project aims to disentangle the mechanisms by which GH25 functions and to study its evolutionary conservation. GH25 of the pathogenic smut fungus *Usilago maydis* , which shows 77% sequence similarity to *MbA* GH25, is being investigated to tackle the evolutionary conservation of GH25 in the context of different fungal lifestyles. Preliminary data implies a comparable inhibition of *Albugo laibachii* by *U. maydis* GH25. The role of *U. maydis* GH25 in its pathogenicity will be further investigated.

Recent evidence suggests the modulation of the microbial community by inhibition of its members as a mode of action of GH25. Indeed, one member of the bacterial community that is closely associated to *A. laibachii* is inhibited by GH25. Moreover, this associated bacterium rescues the inhibition of *A. laibachii* by *MbA* and leads to GH25 upregulation. Ongoing experiments aim to elucidate the biological significance of such a multipartite antagonistic interaction for the inhibition of *A. laibachii* by GH25.

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2-8 Identification and characterization of miR398GGT of *Gaeumannomyces graminis* var. *tritici* and its possible role in plant-fungus interactions

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Wheat is a globally significant crop but is exposed to the Take-all pathogen *Gaeumannomyces graminis* var. *tritici* (GGT), which can cause severe damage in wheat production worldwide. Bidirectional cross-kingdom RNA interference (RNAi) plays important roles in plant–pathogen interactions, in which both plants and pathogens can use small RNAs (sRNAs) to silence target genes. By applying a large-scale metagenomics approach in combination with smallRNA sequencing we identified 9.128 sRNAs from 1.009 fungal genomes, which are potentially involved in interference with wheat gene expression, respectively. Among these, miR398GGT, a homologue of wheat miR398, was found in the wheat roots. Here, we report the identification and characterization of the miR398 locus in the GGT genome and its expression as well as processing during the infection process. First transcript analyses indicate that miR398GGT is likely involved in post-transcriptional silencing SOD target genes in wheat roots. Thus, we assume that miR398GGTmediated suppression of wheat SOD genes may be an essential component of virulence of GGT to evade plant defence response by impairing ROS production. Further investigation on the mode of function of miR398GGT in wheat-fungus

interactions is in progress.

2-9 Adressing redundant roles of phytotoxic proteins for necrotrophic infection of *Botrytis cinerea* by multi-k.o. mutagenesis

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B. cinerea is a wide host range necrotroph. During invasion, it quickly kills host cells and colonizes dead tissue, supported by secretion of CWDE, cell death inducing proteins (CDIPs) and metabolites, and tissue acidification. However, it is still unclear how the fungus induces host cell death. Based on a highly efficient CRISPR/Cas9 protocol, we have constructed a series of up to 26x *B. cinerea* multi-k.o. mutants, lacking all currently known CDIPs (Leisen et al., 2022; unpublished). The mutants showed normal growth, but decreased virulence with increasing numbers of deleted CDIPs. The 26x mutant caused strongly reduced lesion formation on leaves and almost no infection of fruits of different species. Except for the two major polygalacturonases PG1 and PG2, none of the other CDIPs make a major contribution to virulence on most tissues. Genome sequencing and high-resolution secretome analysis of the mutants confirmed loss of the deleted CDIPs. The search for remaining CDIPs is ongoing, to generate finally a non-necrotrophic *B. cinerea* mutant. We are also complementing multi-k.o. mutants with individual overexpressed CDIPs to assess their individual roles for infection of different host tissues. This is one of the first systematic approaches to address functional redundancy of fungal virulence factors.

2-10 Molecular intricacies of smut-induced host resistance toward secondary infection

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The smut pathogen *Ustilago hordei* belongs to the group of Ustilaginales, members of which infect many economically important crops, including maize, wheat, barley, oat, and sugar cane. *S*mut fungi are biotrophic pathogens that depend on living hosts to proliferate. Hence, they need to avoid opportunistic infection of their host. The current study explores, if smuts can actively protect colonized plant parts from secondary infection by other pathogens.

At first, we tested the susceptibility of *Ustilago*-infected host leaves to other pathogenic fungi. We found that maize leaves colonized by *U. maydis* exhibit a strong resistance against the necrotrophic fungal pathogen *Botrytis cinerea*. The similar effect was seen in barley leaves colonized by *U. hordei,* where infection by *B. cinerea* was significantly reduced.

Next, we asked if effectors of *U. hordei* are involved in microbiota manipulation. For this, we performed *in-vitro* induction of effector genes expression and found that this leads to distinct antimicrobial activities of *U. hordei* towards barley-associated bacteria. In future, we will investigate, to which extent microbial antagonisms of *U. hordei* result directly from antimicrobial activity of proteinaceous effectors, or indirectly from the modulation of host defense pathways. A next step will be to use RNAseq to identify effectors of *U. hordei* that are directly involved in the inhibition of competing, barley-associated microbes.

2-11 Searching powdery mildew-resistant oaks for Palatine Forest rejuvenation under climate change

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Oaks (pedunculate and sessile oak; *Quercus robur* and *Q. petraea*) make up ca. 10 percent of the forest tree population in Germany. Because of its ecological and economic value, and its tolerance to warm and dry conditions which are increasingly prevalent during climate change, it is a major silvicultural goal to increase the share of oaks in the Palatine Forest and other German forests. Oak powdery mildew (OPM) caused by *Erysiphe alphitoides* is the most significant oak disease in Europe, and one of the major factors that prevent new oak trees to become established. Recently, the existence of genetically based resistance to powdery mildew in English oak has been documented. The overall goal of the MetaEiche-Projekt ('Identifizierung und Nutzung mehltauresistenter Eichen für die Waldverjüngung im Klimawandel'; funded by the Fachagentur Nachwachsende Rohstoffe e.V. (BMEL) by is to obtain evidences of mildew-resistant oaks, and to promote such oaks during forest regeneration.

By using primers based on the internal transcribed spacer region of 18S rRNA gene, we have found that there is very limited diversity within the OPM population in the Palatine Forest region. Furthermore, to follow the different stages of fungal infection, we have established protocols for staining of OPM on oak leaves with Calcofluor white, Coomassie brilliant blue and wheat germ agglutinin Oregon Green™ Conjugate. In a complementary approach, we are analyzing cytological and molecular markers of resistance. We have optimized an OPM spore suspension inoculation protocol for the study of infection on susceptible and resistance oak using detached leaves. Formation of elongating secondary hyphae (ESH) is a reliable indicator of successful haustorium formation and plant colonization by powdery mildew. Our results show that inoculation of OPM on leaves of partially and fully resistant oak species (*Q. cerris* and *Q. rubra*) results in slightly reduced appressorium formation and strong inhibition of ESH formation compared to the susceptible pedunculate oak, indicating that successful formation of functional haustoria is a crucial step for establishment of the infection. Later stages of OPM infection were also found to be inhibited on leaves of resistant oak species, which

prevented colony formation and conidial reproduction. Our next goal is to use this assay to identify pedunculate oaks showing partial resistance against OPM