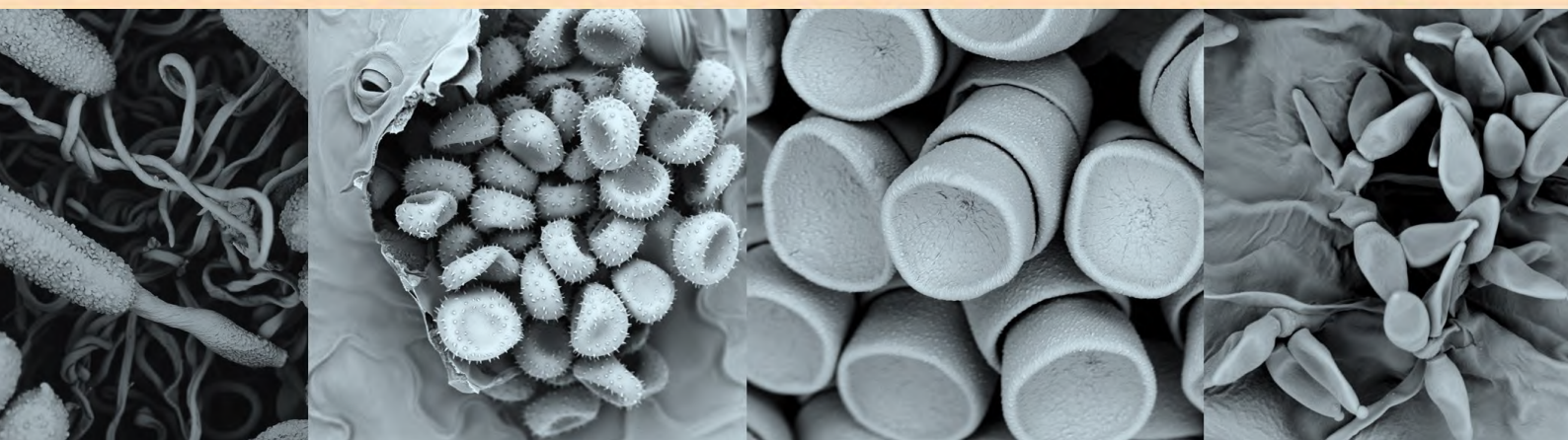


**Gunther Döhlemann (Hrsg.)**

**1. Jahrestagung des DPG-Arbeitskreises  
„Pflanzen Mikrogen Interaktion“ 2025  
an der RPTU Kaiserslautern-Landau**



**Zusammenfassungen der Arbeitskreisbeiträge**

**PI (Persistent Identifier): [urn:nbn:de:0294-jb-ak-2025-pmi-0](https://nbn-resolving.org/urn:nbn:de:0294-jb-ak-2025-pmi-0)**



## **1. Jahrestagung des DPG-Arbeitskreises Pflanzen Mikrogen Interaktion**

**13./14. März 2025**

**an der**

**RPTU Kaiserslautern-Landau**

**Zusammenfassungen der Arbeitskreisbeiträge**

Bericht und Kurzfassungen der DPG Arbeitskreis Tagungen Wirt Parasit Interaktion,  
13./14. März 2025 an der RPTU Kaiserslautern-Landau

Herausgeber: **Gunther Döhlemann**  
**University of Cologne**  
CEPLAS / Institute for Plant Sciences  
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Cover: E.-C. Oerke & U. Steiner - Propagation of *Phragmidium mucronatum*, *Uromyces appendiculatus*,  
*Pustula helianthicola*, *Venturia inaequalis*

PI (Persistent Identifier): urn:nbn:de:0294-jb-ak-2025-pmi-0



Arbeitskreis Pflanzen Mikroben Interaktion

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06.03.2025

## **1. Jahrestagung des DPG-Arbeitskreises „Pflanzen-Mikroben-Interaktionen“ am 13./14. März 2025 an der RPTU Kaiserslautern-Landau**

Liebe Kolleginnen und Kollegen,

in diesem Dokument finden Sie das Programm der Beiträge zur ersten Tagung des neu formierten DPG-Arbeitskreises „Pflanzen-Mikroben-Interaktionen“ am 13./14. März 2025.

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 beibehalten. 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** (Dateiname nach Schema „Beitragsnummer\_Name“, z.B. „1-1\_Ahmad“) **bis spätestens zum 11. März, an [g.doehlemann@uni-koeln.de](mailto:g.doehlemann@uni-koeln.de)**.

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

Mit den besten Grüßen

Gunther Döhlemann und Marco Thines

## PROGRAMM

Donnerstag, 13. März 2025

### 13:00 Uhr BEGRÜßUNG

#### VORTRAGS-SESSION 1 (Leitung: Ulrich Schaffrath)

- 13:10 Uhr Alex Wegner (Aachen): Update on MoNudix; an effector of *Magnaporthe oryzae* which hijacks host phosphate signaling
- 13:30 Uhr Merle Bilstein-Schloemer (Köln): Overlap between virulence and avirulence functions of *Blumeria hordei* AVRA13
- 13:50 Uhr Matthias Kretschmer (Vancouver): Glutathione mediated redox state is important for *Ustilago maydis* growth, melanin formation and virulence
- 14:10 Uhr Shivam Chaudhary (Jena): *Sporisorium reilianum* effector protein SOVIG4 modulates host-specific virulence on sorghum
- 14:30 Uhr Andrea Tobian Herreno (Kiel): *Zymoseptoria tritici* show local differences in within-field diversity and effector variation

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

- 1-1: Farooq Ahmad (Kiel): A genome-wide exploration of defence mechanisms in wild plants using a model tomato species (*Solanum chilense*)
- 1-2: Maria José Ladera Carmona (Gießen): Exogenous dsRNA triggers sequence specific RNAi and fungal stress responses to control *Magnaporthe oryzae* in *Brachypodium distachyon*
- 1-3: Sabrine Nasfi (Gießen): High precision quantification of small RNA slicing activity - native index ligation-based targeted degradome sequencing (NIL-TDS)
- 1-4: Maurice König (Köln): Processing and release of the phyto cytokine Zip1
- 1-5: Nandeesh Jalahalli Rangegowda (Kiel): Genome-wide identification of LRR-RLPs highlights distinct patterns of presence and absence in defence and developmental-related clusters within and between tomato species
- 1-6: Yao Chen (Kiel): Contrasting roles of D-clade ERFs in tomato defense against *Alternaria* pathogens
- 1-7: L. Wirtz (Aachen): Uncovering cell-specific responses of barley cells in response to *Magnaporthe oryzae* infection
- 1-8: Sarah Daher (Köln): Interplay of the fungal endophyte *Fusarium verticillioides* with the pathogenic smut fungi *Ustilago maydis* and *Sporisorium reilianum* in maize
- 1-9: Weiliang Zuo (Köln): Functional adaptation of a transcriptional activator effector family in smut fungi and beyond
- 1-10: Alejandra Vielba-Fernandez (Kaiserslautern): A fight for survival: The role of the NADPH oxidase RbohD in the *Botrytis cinerea* - plant interaction

### **15:15 bis 16:00 Kaffeepause & Postersession 1**

#### **VORTRAGS-SESSION 2 (Leitung: Gunther Döhlemann)**

- 16:00 Uhr Philipp Lopinski (Marburg): The *Ustilago maydis* transcription factor Nit2 controls nitrate assimilation during biotrophy and adjusts organic nitrogen metabolism in maize leaves under N limitation
- 16:20 Uhr Mariana Schuster (Halle): Extracellular proteases from pathogenic fungi and their role in infection
- 16:40 Uhr Sophia Hein (Freising): Multiomics of *Fusarium culmorum* infection in barley
- 17:00 Uhr Jennifer Thielmann (Gießen): Wheat diversity set analyses reveal genotype-specific priming capacities induced by beneficial endophytes
- 17:20 Uhr Janina Werner (Köln): A recombinant smut hybrid provides insights into gene regulation, pathogenesis and tumorigenesis

#### **17:40 Uhr Ehrungen; Wahl der neuen Arbeitskreisleiter**

**ab 18:30 Uhr Gemeinsames Abendessen im Brauhaus an der Gartenschau, Forellenstr. 6, 67659 Kaiserslautern**

### **Freitag, 14. März 2025**

#### **VORTRAGS-SESSION 3 (Leitung: Matthias Hahn)**

- 08:40 Uhr Stefan Thomas (Hohenheim): Monitoring von Apfelanlagen mittels optischer Sensoren und moderner Datenanalyse
- 09:00 Uhr Muhammad Saeed (Kaiserslautern): Oak regeneration in a changing climate: addressing powdery mildew threats
- 09:20 Uhr Ana Sede (Strasbourg): Development of RNA-based bioprotectants against viruses for sustainable crop production
- 09:40 Uhr Sabine Kind (Dossenheim): Fungal communities in sea buckthorn in context of its dieback
- 10:00 Uhr Yixuan Yang (Göttingen): Whole genome sequencing reveals population structure of *Cercospora beticola* to resistant sugar beet cultivars in Germany

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

- 2-1: Pelias Rafanomezantsoa (Hohenheim): *Bacillus halotolerans* B21 effective against *Fusarium graminearum* and *Erwinia amylovora* - potential use as a biocontrol agent
- 2-2: Wei Shi (Köln): Identification of AVRA effector targets in barley
- 2-3: Christina Steidele (Freising): Network inference reveals distinct transcriptional regulation patterns in barley
- 2-4: Andrea O'Reilly (Köln): Characterisation of conserved virulence factors of *Puccinia sorghi* and *Ustilago maydis*

- 2-5: Lukas Dorian Dittiger (Jena): The host-specificity factor SOVIG9 of *S. reilianum* affects phytoalexin induction in *Sorghum bicolor*
- 2-6: Rama Krishna Konda (Jena): Deciphering the molecular mechanism of a fungal effector protein that impairs plant development
- 2-7: Nassim Safari (Kaiserslautern): Addressing redundant roles of phytotoxic proteins for necrotrophic infection of *Botrytis cinerea* by multi-k.o. mutagenesis
- 2-8: Marco Loehrer (Aachen): Exploring potential benefits of biostimulant treatments in lupin cultivation
- 2-9: Yvonne Becker (Braunschweig): Interbacterial competition by R-type phage tail-like particles
- 2-10: Sonja Raetz (Braunschweig): Application of beneficial bacteria as an induced resistance-based approach for enhanced resilience
- 2-11: Tobias Müller (Kaiserslautern): Manipulation of plant immunity by the fungal pathogen *Botrytis*

### **10:45 bis 11:30 Kaffeepause & Postersession 2**

#### **VORTRAGS-SESSION 4 (Leitung: tba)**

- 11:30 Uhr Zarah Sorger (Köln): Exploring the role of GH25 in fungal lifestyle and microbial antagonism
- 11:50 Uhr A. Y. Rudolph / Daniela Nordzieke (Göttingen): Deciphering the interactions between *Colletotrichum graminicola* and maize roots
- 12:10 Uhr K. Omengé / Stephanie Werner (Quedlinburg): LysM receptor function in successful ectomycorrhiza formation between poplar and *Laccaria bicolor*
- 12:30 Uhr Marion Müller (Freising): Avirulence depletion assay: combining R gene-mediated selection with bulk sequencing for rapid avirulence gene identification
- 12:50 Uhr Bernhard Werner (Gießen): Native sRNAs in plant protection

**13:20 Uhr: Bekanntgabe Ort und Termin der nächsten Arbeitskreistagung;  
Verabschiedung**

# **Abstracts oral presentations**

## Update on *MoNudix*; an effector of *Magnaporthe oryzae* which hijacks host phosphate signaling

Wegner, A.<sup>2</sup>, McCombe, C. L.<sup>1</sup>, Wirtz, L.<sup>2</sup>, Zamora, C. S.<sup>3</sup>, Casanova, F.<sup>2</sup>, Aditya, S.<sup>1</sup>, Greenwood, J. R.<sup>1</sup>, de Paula, S.<sup>3</sup>, England, E.<sup>1</sup>, Shang, S.<sup>1</sup>, Ericsson, D.J.<sup>4</sup>, Oliveira-Garcia, E.<sup>3</sup>, Williams, S.J.<sup>1</sup>, Schaffrath, U.<sup>2</sup>

<sup>1</sup>Research School of Biology, The Australian National University; Canberra, 2617, Australia

<sup>2</sup>Department of Molecular Plant Physiology, RWTH Aachen University; Aachen, 52056, Germany.

<sup>3</sup>Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center; Baton Rouge, 70803, United States of America.

<sup>4</sup>ANSTO, Australian Synchrotron, Crystallography Beamline Group; Melbourne, 3168, Australia.

To successfully infect host plants, phytopathogenic fungi have evolved sophisticated mechanisms to suppress plant immune responses. Here, we highlight a conserved family of small secreted effectors known as Nudix-proteins, which are present across multiple phytopathogenic fungi, such as *Magnaporthe oryzae*, *Colletotrichum higginsianum* and *C. graminicola* and play a crucial role in host colonization.

Our study explores how these effectors interact with the plant phosphate starvation pathway to promote infection. Gene deletion mutants of the Nudix effectors were generated in *M. oryzae*, *C. higginsianum* and *C. graminicola* to assess their impact on virulence. In all tested pathosystems, the Nudix proteins were found to be essential for full virulence, pointing to a conserved mechanism. Complementation experiments in *M. oryzae* Nudix-gene deletion mutants using respective proteins from *Colletotrichum*, as well as inactive variants, further demonstrated functional redundancy within this effector family and highlighted the importance of the enzymatic activity.

Further, we identified inositol pyrophosphates as the substrates of Nudix effectors. Notably, plants regulate their phosphate status via these molecules, which serve as sensors of inorganic phosphate availability. Depletion of these molecules, as triggered by the activity of fungal Nudix proteins during infection, stimulate the plant's phosphate starvation response, which in turn resulted in suppression of resistance gene expression. RNA sequencing experiments point to the regulatory network associated with Nudix effector activity and provide new insights into fungal pathogenicity strategies.

# Overlap between virulence and avirulence functions of *Blumeria hordei* AVR<sub>A13</sub>

Bilstein-Schloemer, M.<sup>1</sup>, Shi, W.<sup>1</sup>, Hofmann, M.<sup>1</sup>, Saur I. ML.<sup>1,2</sup>

<sup>1</sup>*Institute for Plant Sciences, University of Cologne*

<sup>2</sup>*The Cluster of Excellence on Plant Sciences (CEPLAS)*

The powdery mildew fungus *Blumeria hordei* (Bh) secretes effectors that manipulate barley host components to promote fungal virulence (effector virulence function). Resistance to Bh is often mediated by Mildew locus A immune receptors (MLAs) that recognize Bh AVRA effectors to trigger immune responses that are associated with host cell death (effector avirulence function). Although AVRA13 is recognized by MLA13, AVRA13 is highly conserved in the Bh population.

To determine why AVRA13 remains conserved in the Bh genome despite its avirulence activity, we first set out to understand AVRA13 virulence activity. We identified the barley homolog of the Strubbelig Receptor Family 3 (SRF3) receptor-like kinase as specific interactor of AVRA13. SRF3 is highly conserved across monocots and dicots and AVRA13 can also bind to it in Arabidopsis, where AtSRF3 was shown to regulate iron homeostasis. We detected srf3-dependent and AVRA13-specific effects on the expression of iron deficiency markers in *A. thaliana* and AVRA13-expressing lines are immunodeficient. The data underline that AVRA13 virulence activity likely involves the modulation of SRF3 function.

By generating an extensive set of chimeric and mutant AVRA13 constructs, we demonstrate that a central AVRA13 loop region (aa 44 to 70) mediates the AVRA13 - SRF3 interaction. We also show that the same region is required for activation of MLA13-mediated cell death. Thus, MLA13 has likely evolved to specifically bind the residues of AVRA13 required for its virulence function on SRF3.

Together our data can explain why AVRA13 is maintained in the global Bh population despite being recognized by MLA13 and underlines that Bh cannot diversify AVRA13 to escape recognition by MLA13 without compromising its ability to modulate SRF3 activity.

# **Glutathione mediated redox state is important for *Ustilago maydis* growth, melanin formation and virulence**

Kretschmer, M.<sup>1</sup>, Damoo, D.<sup>1</sup>, Heimel, K.<sup>2</sup> and Kronstad, J.<sup>1</sup>

<sup>1</sup> *The University of British Columbia, Vancouver, Canada*

<sup>2</sup> *Georg-August-Universität Göttingen, Goettingen, Germany*

*Ustilago maydis* is the causal agent of corn smut disease. We recently showed that a combination of glucose plus the organic acid malate (G+M) activated the genetic biotrophy program in *U. maydis*. Observed phenotypic changes in G+M included accelerated growth, extracellular matrix and melanin formation usually only observed during sporulation. The glutathione biosynthesis pathway and the iron-regulon regulator Grx4, which has iron-sulfur clusters and GSH as cofactors, were differently regulated in G+M. Thus, we deleted the glutathione synthetase (Gsh2) and analyzed its impact on growth, melanin formation, stress response and virulence of *Ustilago maydis*.  $\Delta$ gsh2 showed a growth defect on glucose, which could partially be rescued by addition of iron, malate and reduced GSH (GSHred). This links iron and glutathione metabolism with growth on G+M. Surprisingly, 0.5 mM GSHred was toxic to *Ustilago* and induced oxygen dependent melanin formation in glucose medium comparable to Gsh1 inhibition by BSO. The  $\Delta$ gsh2 mutant further showed sensitivity to heat, high pH and ROS. ROS is mainly formed during respiration and the toxicity of GSHred could be linked via Antimycin A to the mitochondrial electron transport chain complex III, while  $\Delta$ gsh2 itself showed severe sensitivity to KCN induced complex IV inhibition. The  $\Delta$ gsh2 mutant further showed reduced virulence which could be rescued by low concentration of GSHred. Interestingly, GSH metabolism inhibition with BSO as well as high concentrations of GSHred also reduced virulence of *Ustilago maydis*. In conclusion we link redox state via ETC functions to growth, melanin formation and virulence in *U. maydis*.

## ***Sporisorium reilianum* effector protein SOVIG4 modulates host-specific virulence on sorghum**

Chaudhary, S.<sup>1</sup>, Dittiger, L.D.<sup>1</sup>, Bhawna, B.<sup>1</sup>, Hernandez, J.A.R.<sup>1</sup>, Schirawski, J.<sup>1</sup>

<sup>1</sup>Friedrich Schiller University, Department of Genetics,  
Email: shivam.chaudhary@uni-jena.de

*Sporisorium reilianum* is a biotrophic basidiomycetous fungus responsible for head smut disease in maize and sorghum. This soil-borne pathogen exists in two distinct formae speciales: *S. reilianum* f. sp. *reilianum* (SRS) and *S. reilianum* f. sp. *zeae* (SRZ). Both can infect and penetrate the leaves of both maize and sorghum, but each can only cause smut disease on its respective host—SRS on sorghum and SRZ on maize. To investigate the molecular mechanisms behind this host-specificity, we employed a classical genetics approach, combining hybridization with next-generation sequencing (NGS) and genome-wide association studies (GWAS) analysis. This led to the identification of an effector cluster whose origin from SRS was associated with the spore-forming ability of SRS in sorghum. Deletion of this cluster in SRS resulted in a significant reduction in virulence on sorghum. Additionally, we created knockout strains lacking individual cluster genes. Virulence assays showed that the deletion of *SOVIG4* contributes most to the disease phenotype of *Sporisorium reilianum*. We found out that SOVIG4 localised in the cytoplasm and nuclei of plant cells when transiently expressed in the *Nicotiana benthamiana*. To understand the mechanism of effector protein function, we did comparative RNA-sequencing and metabolome analysis of wildtype- and knockout-strain inoculated sorghum leaves. Additionally, we did a yeast two-hybrid screening for the identification of putative interaction partners from a *Sporisorium reilianum*-infected sorghum cDNA library. Based on the findings, we predict that SOVIG4 has a function in suppressing early plant defense responses. We will present a model of the role of SOVIG4 in host-specific virulence of *S. reilianum* f. sp. *reilianum* on sorghum.

## ***Zymoseptoria tritici* show local differences in within-field diversity and effector variation**

Tobian Herreno, A.<sup>1</sup>, Huang, P.<sup>2</sup>, Siepe, I.<sup>3</sup>, Stam, R.<sup>1\*</sup>

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*Zymoseptoria tritici*, a cosmopolitan hemibiotrophic wheat pathogen, poses significant challenges to traditional farming management due to its high mutation rate and mixed reproduction system, involving both sexual and asexual cycles within the same disease cycle. Understanding population diversity in the field is crucial for effective pest management and early detection of new aggressive lineages. This study examines whole-genome sequence data from three datasets—one from a newly sampled field in the United Kingdom and two publicly available datasets from the United States and Switzerland—to assess within-field diversity.

Our analysis of population structure, minor allele frequency distribution, and clonality reveals no distinct within-field structure, with abundant SNPs occurring at low frequencies and higher clonality in European fields. Regional population separation is evident at the whole-genome scale, but no such separation is observed in effector genes. Interestingly, multiple effector haplotypes are interspersed within fields, showing no private haplotypes among various avr genes like *avrstb6*, *avrstb9* and *avr3D1*. Furthermore, within clonal clusters we also observe multiple effector haplotypes for effectors involved in the biotrophic and necrotrophic phases of the disease.

This high level of within-field genetic diversity, particularly in effector genes, could explain *Z. tritici*'s significant potential to overcome resistance genes. Our findings suggest that the diversity within field populations of *Z. tritici* is greater than previously recognised, highlighting the complexity of managing this pathogen.

# **The *Ustilago maydis* transcription factor Nit2 controls nitrate assimilation during biotrophy and adjusts organic nitrogen metabolism in maize leaves under N limitation**

Lopinski, P.L., Schulz, C., Fischer, A., Reichl, N., Engelsdorf, T., Braun, N., Voll, L.M.

*Philipps-Universität Marburg*

Little is known on how filamentous phytopathogens adapt nitrogen acquisition upon nitrogen limitation in the host plant. In previous work, we have shown that the transcription factor Nit2 plays a major role for the utilization of non-favored nitrogen sources like nitrate, minor amino acids or nucleobases in saprotrophic sporidia of the basidiomycete corn smut fungus *Ustilago maydis*. Here, we employed  $\Delta$ nit2 mutants in the natural FB1 x FB2 background to identify Nit2 target genes during biotrophy and investigated the impact of Nit2 on the physiology of leaf galls in nitrogen replete versus nitrogen limited host plants. Comparative RNA-Seq analysis of galls caused by the *U. maydis*  $\Delta$ nit2 mutant and by the wild type, revealed that about one third of Nit2 targets during biotrophy are involved in nitrogen metabolism and transport. Induction of the nitrate assimilation cluster was completely dependent on Nit2, which lead to substantial accumulation of nitrate and reduced accumulation of the nitrogen-rich phloem transport amino acids asparagine and glutamine in  $\Delta$ nit2 leaf galls compared to wild type galls under nitrogen limitation, while diminished asparagine contents were the only physiological difference in  $\Delta$ nit2 leaf galls in nitrogen replete control plants. Since total protein content in galls and pathogenicity were comparable between fungal genotypes in both nitrogen regimes, the findings of our physiological and transcriptomic analysis demonstrate that nitrate utilization is dispensable for *Ustilago maydis* during biotrophy and can likely be actively compensated by increased utilization of abundant organic nitrogen sources, like asparagine, GABA and glutamine in a partially Nit2-dependent fashion.

## **Extracellular proteases from pathogenic fungi and their role in infection**

Vijayan, V. and Schuster, M.

*Receptor Biochemistry Group, Leibniz Institute of Plant Biochemistry, 06120 Halle (Saale), Germany*

Plant pathogens secrete specialized proteins to facilitate infection, including extracellular proteases that function in the apoplast. These virulence proteases play key roles in plant cell wall degradation and immune evasion. However, despite the abundance of predicted extracellular proteases in plant pathogens, their characterized functions remain limited, especially compared to the well-studied diversity of extracellular proteases in animal pathogens. To bridge this gap, we have identified and characterized the extracellular protease repertoire of the rice blast fungus *Magnaporthe oryzae*, analysing its composition, conservation, and expression patterns. Our findings provide insights into the potential roles of these proteases in fungal virulence.

## **Multionics of *Fusarium culmorum* infection in barley**

Hein, S.; Steidele, C.; Hoheneder, F.; Hüchelhoven, R.

*Chair of Phytopathology, TUM School of Life Sciences, Technical University of Munich*

Fusarium Head Blight is a devastating fungal disease in small grain cereals like wheat and barley, leading to worldwide yield losses every year. These issues are becoming more pressing due to climate change and a growing global population. While barley was shown to react to Fusarium infection with transcriptional defense responses, mycotoxins like Deoxynivalenol can impair protein biosynthesis. It remains unclear how effectively immune-related transcripts are successfully translated into proteins. To date, no study has explored the molecular response to FHB in barley on an in-depth multiomics level. We investigated how gene regulation in barley during Fusarium culmorum infection translates to protein and metabolite levels, aiming to identify infection-related metabolic pathways. Our results identified a subset of significantly regulated gene-protein pairs associated with biosynthetic pathways linked to upregulated metabolites. Notably, this includes metabolites known for their roles in pathogen defense like tryptophan and serotonin as well as barley specific compounds like hordatines and their precursors. Our findings provide new insights into the complex interactions between barley and Fusarium, shedding light on key metabolic processes involved in pathogen defense.

# Wheat diversity set analyses reveal genotype-specific priming capacities induced by beneficial endophytes

Thielmann J<sup>1</sup>, Soleimani B<sup>2</sup>, Wehner G<sup>2</sup>, Matros A<sup>2</sup>, Schacht J<sup>3</sup>, Wiegmann M<sup>4</sup>, Ordon F<sup>2</sup>, Kogel KH<sup>1</sup>, Schäfer P<sup>1</sup>

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Beneficial microorganisms can trigger a response in plants named priming. Priming is a durable condition allowing plants a fast and effective immune response against pathogens. Additionally, it often translates into increased biomass and yield. A plant's ability to reach this condition is defined as priming capacity. The genetics of this phenomenon are still unknown, but a deeper understanding and breeding for priming-responsive crops could contribute to reducing the use of pesticides and fertilizers. In this study, we analyzed the priming responses of a genetically diverse set of selected winter wheat (*Triticum aestivum*) genotypes. Two beneficial fungal and bacterial endophytes, *Serendipita indica* and *Rhizobium radiobacter* F4, served as priming inducers. Priming treatment effects on diverse traits were evaluated in different experimental setups. A screening of the whole diversity set revealed high genotype dependency for endophyte-mediated priming responses. Genome-wide association studies (GWAS) identified several quantitative trait loci (QTLs) linked to priming effects on biomass and induced resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*). Some candidate genes in these QTL regions are involved in plant defenses and priming-related signaling pathways. Further experiments under semi-field and field conditions indicated agronomic applicability of microbe-induced priming as effects on plant development and resistances could be observed under environmental impacts.

# A recombinant smut hybrid provides insights into gene regulation, pathogenesis and tumorigenesis

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The maize smut fungi *Ustilago maydis* and *Sporisorium reilianum* are closely related and share similar genome sizes and synteny. *U. maydis* causes localized tumor formation at the infection site, whereas *S. reilianum* spreads systemically throughout the host and induces symptoms in the inflorescences, without obvious symptoms during the early infection phase. To investigate the molecular basis of these phenotypic differences in maize, we generated an interspecific recombinant hybrid (rUSH) that contains the mating type system of *S. reilianum*. rUSH exhibited extensive *in planta* growth and displayed a phenotype similar to *S. reilianum* at all stages of development, except teliospore formation. Transcriptome analysis revealed that pathogenicity-related effector orthologs were up-regulated in rUSH, but not in a wild-type hybrid control. We found 253 differentially expressed one-to-one effector orthologs in rUSH, with distinct regulatory patterns, including *cis*-, *trans*-, and rUSH-specific regulation. Using CRISPR/Cas9-mediated mutagenesis, we identified three novel virulence factors associated with the rUSH-specific effector regulation.

Ultimately, rUSH led to the identification of the transcription factor *UmHdp2* as a key regulator of tumorigenesis and a subset of 41 putative tumorigenic effector genes. These findings demonstrate the potential of interspecific recombinant hybrids to study the molecular mechanisms underlying pathogenic differences in closely related fungi.

# Monitoring von Apfelanlagen mittels optischer Sensoren und moderner Datenanalyse

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Der Apfelanbau steht momentan vor großen Herausforderungen. Einerseits wünschen Politik und Gesellschaft eine erhebliche Reduktion des Einsatzes chemisch-synthetischer Pflanzenschutzmittel – bis zu 50% in 2030 – andererseits kommen durch den Klimawandel neue und nicht ausreichend untersuchte Umweltfaktoren zum Tragen, welche gerade auf den langfristigen Apfelanbau erhebliche Folgen haben könnten. Optische Sensoren – in Verbindung mit moderner Datenanalyse – stellen einen möglichen Lösungsansatz dar. Die ausgewerteten Sensordaten unterstützen Landwirte bei der Entscheidungsfindung indem biotische und abiotische Stressfaktoren frühzeitig erkannt werden und somit der Einsatz von Pflanzenschutzmitteln erheblich reduziert werden kann. Innerhalb der vorgestellten Studie wurden hyperspektrale Sensoren zum Monitoring von Apfelanlagen unter Praxisbedingungen am Kompetenzzentrum Obstbau Bodensee (KOB) von April bis September verwendet und die entsprechenden Datensätze ausgewertet. Es war möglich sowohl biotische (Pathogenbefall, Insektenschäden) als auch abiotische (Blattläsionen) Faktoren zu detektieren und klar voneinander zu unterscheiden. Um dies zu erreichen wurden die Daten mittels verschiedener Machine Learning Methoden in mehreren Schritten analysiert. Zuerst wurde der durch die Gegebenheiten in der Apfelanlage komplexe Hintergrund entfernt um anschließend die verschiedenen symptomatischen Bereiche anhand von durch Experten erstellte Trainingsdaten zu klassifizieren. Obwohl die Methode noch weiter ausgereift werden muss, um tatsächlich in der landwirtschaftlichen Praxis einsetzbar zu sein, zeigen die Ergebnisse der Studie, dass eine gezieltere Anwendung von Pflanzenschutzmitteln durch die gegebenen Entscheidungshilfen möglich wäre.

# Oak Regeneration in a Changing Climate: Addressing Powdery Mildew Threats

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The MetaEiche project, funded by the Federal Ministries BMEL/BMU through the Waldklimafonds, aims to minimize damage caused by powdery mildew during the regeneration of oak stands. Native oaks (*Quercus robur* and *Q. petraea*) constitute approximately 10% of Germany's forests and are among the most valuable tree species. Due to their superior drought resilience, oaks are expected to play a crucial role in climate-adapted forest management, making their successful regeneration a long-term silvicultural goal. A significant challenge in oak regeneration is infestation by oak powdery mildew (OPM), which weakens young oaks and threatens regeneration success. Notably, varying levels of susceptibility have been observed in native oaks, suggesting the presence of partial resistance. The project seeks to identify and leverage genetically determined resistance mechanisms to enhance the resilience of future oak stands.

To quantitatively evaluate OPM sensitivity or resistance, an infection assay with detached oak leaves was developed. With this assay, species-specific differences in OPM resistance were revealed. Sessile oak (*Q. petraea*) was highly susceptible, promoting rapid fungal colonization. Turkey oak (*Q. cerris*) displayed partial resistance, characterized by restricted hyphal development and the absence of mature spore-producing structures. Red oak (*Q. rubra*) exhibited near-complete resistance, with fungal progression halted at the early stages of infection (supporting the concept of nonhost resistance, as discussed below).

Studies in forest oak stands and demonstration plots in the Palatinate Northern Vosges were performed to monitor powdery mildew incidence and to assess key physiological parameters in the leaves, including phenol and tannin content, oxidative stress markers, and antioxidant activity. A plantation trial at Antonihof (2020–2023) identified Rheingrafenstein (RGS) as consistently resistant and Heidenkopf (HK) as susceptible to OPM. Detached leaf assays conducted in 2024 quantified mycelium development at 3 dpi, revealing a difference in mean mycelium area between RGS and HK, though not statistically significant. These findings represent preliminary data. Although these findings suggest resistance differences, further investigation is needed for confirmation.

In 2024, five cases of OPM were observed on *Q. rubra* in Kaiserslautern, Germany. Molecular analysis identified *E. alphitoides* or *E. quercicola*. Controlled inoculations showed minimal pathogen development, indicating nonhost resistance. While *Q. rubra* still remains resistant, these findings raise concerns that a changing climate may increase its susceptibility to OPM and further exacerbate infections in native oak species. The presentation will provide an update on the current progress of the project and its implications for sustainable oak regeneration.

# Development of RNA-based bioprotectants against viruses for sustainable crop production

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The European ERA-NET SusCrop Project **BioProtect** aims to contribute to reducing the application of chemical pesticides through the development and cost-effective production of nature-friendly dsRNA-based bioprotectants. dsRNA is a natural and environmentally safe molecule that originates from RNA interference (RNAi) pathways involved in gene regulation and pathogen defense. Its exogenous application to plants leads to the activation of RNAi which can be programmed against specific pathogens depending on the designed dsRNA nucleotide sequence. In addition, dsRNA has the potential to trigger pattern-triggered immunity (PTI) thereby reinforcing crop protection through a second host defense pathway. In this project, we use a bacterial dsRNA-production platform that employs the RNA-dependent RNA polymerase of the dsRNA bacteriophage Phi6 to produce large amounts of high-quality (hq, fully duplex aligned) long-dsRNA with homology to specific viruses such as TMV or TuMV. After purification, the hq-dsRNAs are formulated into biopolymer nanoparticles for enhanced stability and foliar uptake. Our results indicate that our formulation protects hq-dsRNA against RNAses and that hq-dsRNA application provides local and systemic antiviral protection over several days following treatment. Our current studies are aimed at optimizing methods for hq-dsRNA delivery by spraying.

# Fungal communities in sea buckthorn in context of its dieback

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During recent years, German sea buckthorn is increasingly affected by dieback. Both habitats, wild plants on dunes and cultivated plants grown in plantations, display symptoms of leaf yellowing, bark lesions, wilt, and dieback. As result from an extensive isolation approach, *Hymenopleella* and *Diaporthe* were the most frequently isolated fungal genera from symptomatic shoot samples. In addition to the isolation approach, a culture-independent sequencing approach was applied to identify potential pathogens that are causing or contributing to the disease including those fungi that are recalcitrant to isolate. The results confirm the dominance of *Diaporthe* and *Hymenopleella* in symptomatic tissue. Furthermore we tested sea buckthorn planting material for these fungal genera and identified tissue age and position as the most crucial factor for fungal community composition rather than cultivar or origin.

# Whole genome sequencing reveals population structure of *Cercospora beticola* to resistant sugar beet cultivars in Germany

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*Cercospora* leaf spot (CLS), caused by the fungal pathogen *Cercospora beticola*, is the most destructive sugar beet foliar disease. Management of CLS relies heavily on fungicide applications, however the emergence of fungicide-resistant populations emphasizes the importance of developing resistant cultivars. Understanding the interactions between cultivar resistance and *C. beticola* is essential for sustainable CLS management. To gain better insight into the structure of *C. beticola* populations collected from cultivars bearing distinct resistance properties, field trials were conducted with four sugar beet cultivars with different resistance properties in four geographical locations in Germany in 2022 and 2023. Experimental plots were inoculated with CLS-infested leaf material in 2022. Leaf material from each variety was harvested separately at the end of the season as inoculum for 2023. A population of 25 *C. beticola* isolates was obtained from each cultivar at each location, resulting in a total number of 800 isolates in both years. Isolates were subjected to whole genome sequencing and variant discovery to perform population genomic analysis. Principal component analysis showed little variation among populations obtained from different locations or cultivars, although location-specific lineages were identified in both years from one of the trial locations. Population genomic scans revealed eight genomic regions associated with certain host genotypes. Further experiments will focus on validating the functions of genes underlying these associated regions.

# Exploring the Role of GH25 in Fungal Lifestyle and Microbial Antagonism

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Plants are colonized by a variety of microorganisms that not only interact with their host, but also exhibit 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 negative (1).

The pathogenic oomycete *Albugo laibachii* has been identified as a hub for shaping the microbial community. The basidiomycete yeast *Moesziomyces bullatus ex Albugo on Arabidopsis (MbA)* inhibits *A. laibachii* and significantly reduces its virulence (2). RNA sequencing was performed to discover genes involved in the inhibition of *A. laibachii*, which identified putative glycoside hydrolase (GH25). Deletion of the gene resulted in an almost complete loss of antagonistic activity of *MbA* against *A. laibachii* (2).

This project aims to decipher the mechanism of action of GH25 and to study its evolutionary conservation in plant-colonizing fungi. GH25 of the pathogenic smut fungus *Ustilago maydis* was studied to investigate the evolutionary conservation of GH25 in the context of different fungal lifestyles. We established a strain collection for the phyllosphere of *Zea mays* and identified “health-relevant” bacteria therein. These bacteria proved to be targets of *U. maydis* GH25, and co-inoculation with these bacteria reduced the virulence of *U. maydis* in the mutant strain  $\Delta$ GH25. Thus, in *U. maydis* GH25 acts as an antimicrobial effector that increases pathogen fitness. To decipher the mechanism by which GH25 mediates antagonism between *MbA* and *A. laibachii*, we tested whether it could act by modulating the microbial community associated with *A. laibachii*. Indeed, we found that GH25 inhibits the bacteria associated with *A. laibachii*. In turn, the same bacteria can override the inhibition of *A. laibachii* by *MbA* and lead to an upregulation of GH25. To link these results to our interest in evolutionary conservation, we compared the target specificity of GH25 of the orthologs of *MbA*, *U. maydis* and *Rhizoctonia solani*. We found that only GH25 from *MbA* and *U. maydis* was able to inhibit bacteria associated with *A. laibachii* and in turn inhibit *A. laibachii* on planta. Taken

together, our results suggest a tripartite antagonistic interaction responsible for the inhibition of *A. laibachii* by GH25.

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# Deciphering the interactions between *Colletotrichum graminicola* and maize roots

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*Zea mays* is the most grown cereal for human and animal nutrition, making detailed knowledge about maize-pathogen interactions essential for food security. The hemibiotrophic fungus *Colletotrichum graminicola* is a maize pathogen responsible for annual harvest losses of 10-20% worldwide. In previous work we showed that *C. graminicola* generates two distinct asexual spore types, oval and falcate conidia, which exhibit specialized leaf infection strategies determined by spore-type-specific secretion patterns. In recent root infection experiments we found that only oval but not falcate conidia provoke stunting of maize plants and propagation of the fungus into the stem. As described for other root pathogenic fungi, we speculated about host-guided direct growth of the pathogen prior to root infection. Applying a combination of HPLC/MS analysis and biological assays, we indeed identified diterpenoids as part of the maize root exudate (MRE) provoking chemotropic growth of *C. graminicola* oval conidia. In a second step, we aimed to identify *C. graminicola* genes responsible for diterpenoid sensing. Characterization of deletion mutants provided evidence that diterpenoids are perceived via the  $\alpha$ -pheromone receptor CgSte3, activating the downstream cell wall integrity MAPK pathway (CWI), resulting in growth re-direction. Since homologous components are required for the sensing of plant peroxidases by other root interacting fungi, we tested the same strains for their ability to sense peroxidases. Intriguingly, we found that *C. graminicola* oval conidia do not only grow chemotropically to peroxidase gradients but also the corresponding perception is routed via CgSte3 and the CWI. These last results provoke the question, whether plant-recognition by root exudate sensing is as host-specific as generally assumed.

# **LysM receptor function in successful ectomycorrhiza formation between poplar and *Laccaria bicolor***

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The colonization of plants by symbiotic fungi is highly regulated by both partners. For successful symbiosis, interspecies communication depends on the interaction of specific receptors on the plant side and signaling molecules of the fungus to induce the common symbiosis signaling pathway (CSP) in the host to induce an array of genes needed for symbiosis initiation. In this context, the plant must distinguish between a symbiotic and a pathogenic fungus. This is mediated by the specificity of the receptors of the host and the fungal molecules activating them. Membrane-associated chitin binding LysM receptor proteins initiate intracellular signaling events that either induce defense against pathogens or allow symbiosis. For many pathogens, symbiotic bacteria, and arbuscular mycorrhizal fungi, receptor candidates of this class are described to be crucial for signal transmission. For the formation of ectomycorrhizal (EM) associations of symbiotic soil fungi with tree hosts, receptors are unknown. In this cooperative project, we are characterizing three LysM receptor candidates of poplar (*Populus spec.*) that might be crucial for EM formation with the fungus *Laccaria bicolor*. To understand their function for symbiosis initiation, we produced knockout mutants of all receptors (single-, double- and triple mutants). We investigated the activation of the CSP, downstream gene expression, and mycorrhiza formation. Visualization of nuclear calcium spiking as readout for CSP induction demonstrated that at least two of these three receptors seem to be required for signal transmission since spiking was highly reduced in triple and double mutants compared to wild type plants. Furthermore, induction of gene expression downstream in the CSP was impaired. Microscopic evaluation of wild type and mutant mycorrhized root tips also showed significant differences. Especially in double and triple mutants, we observed reduced mycorrhiza formation and partially deformed root tip structures than in wild type co-cultivations demonstrating the importance of these receptor candidates for EM

formation. In summary, our findings support the hypothesis that EM fungi use LysM receptor-mediated signaling pathways to establish a symbiotic association with the roots of woody plants.

# Avirulence depletion assay: Combining *R* gene-mediated selection with bulk sequencing for rapid avirulence gene identification

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Wheat production faces significant threats from various fungal pathogens. Resistance breeding in wheat often depends on the utilization of resistance (*R*) genes that encode diverse immune receptors capable of detecting specific avirulence (*AVR*) effectors, thereby triggering an immune response. While the cloning of *R* genes has advanced in recent years, the identification of *AVR* genes in many fungal pathogens, including *Blumeria graminis* f.sp. *tritici* (*Bgt*), lagged behind. As a result, the strategic deployment of these resistance sources, informed by pathogen diversity studies, remains underexplored. To address this issue, we developed an new “avirulence depletion (*AD*) assay” designed for the rapid identification of *AVR* genes in *Bgt*. This assay involves selecting a segregating haploid F1 progeny population on a resistant host, followed by bulk sequencing, thereby allowing for rapid identification of avirulence candidate genes with high mapping resolution. The *AD* assay represents a powerful and cost-effective tool for the rapid identification of *AVR* genes in *Bgt*, with the potential to enhance pathogen-informed breeding strategies for employing novel *R* genes and regionally tailored gene deployment against *Bgt* and other pathogens.

## Reference

Kunz L, Jigisha J, Menardo F, Sotiropoulos AG, Zbinden H, Zou SH, Tang DZ, Hückelhoven R, Keller B, **Müller MC**. 2025. Avirulence depletion assay: Combining *R* gene-mediated selection with bulk sequencing for rapid avirulence gene identification in wheat powdery mildew. *Plos Pathogens* 21(1). 10.1371/journal.ppat.1012799

## Native sRNAs in plant protection

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RNA interference (RNAi) in crop protection is an emerging technology. Synthetic RNAi-based products have been already approved indicating their high potential for disease and pest control in future. The advantage of RNAi is the high specificity based on the complementarity of small RNAs together with short synthesis cycles. In turn, we lack an exact understanding about the predictability of RNAi efficiency, potential off-target effects or any dynamics in the development of resistances.

To address these problems, our aim is to exploit the untapped potential of natural plant sRNAs. As part of a process called cross-kingdom-RNAi (ckRNAi), plants release sRNAs into pathogens to prevent infection and disease development. To this end we conducted a deep and improved degradome, sRNAome and transcriptome sequencing of *Brachypodium distachyon* (*Bd*) in interaction with the pathogens *Fusarium graminearum* (*Fg*), *Pyricularia oryzae* (*Po*) and three *Puccinia* species. This extensive ckRNAiome dataset was analysed via a newly developed ckRNAi analysis pipeline. The simulation of random degradomes allowed to elucidate specific sRNA classes of defined sizes and 5'-terminal nucleotides (nt), responsible for ckRNAi.

A total of 43 *Bd*-anti*Fg*-sRNAs were confirmed under highly stringent selection criteria. Interestingly, we found *Bd*-derived sRNAs targeting fundamental metabolic processes in *Fg* to originate from a long non-coding RNA (lncRNA). Phasing of sRNAs from this lncRNA indicates a biogenesis via the tasiRNA pathway. Most importantly, spray-application of *Bd*-anti*Fg*-sRNA16 successfully protected *Bd* from *Fg* infection at exceptionally low sRNA concentrations. We further predict sRNAs of this type to also be effective against a broad spectrum of phytopathogens, while still being very specific to fungi.

# **Abstracts poster pitches**

## **1-1 A genome-wide exploration of defence mechanisms in wild plants using a model tomato species (*Solanum chilense*)**

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Wild plants are regularly exposed to different biotic and abiotic stresses, which put them under constant need to evolve. Through evolution, they develop new traits and adapt to different habitats. In this study, we sequenced whole-genomes of 99 individuals of a wild tomato species (*Solanum chilense*) from nine populations representing three genetic clusters and recorded several stress-related phenotypes before and after infecting with *Alternaria alternata*. The quantitative phenotypes included external phenotypes (quantitative resistance, leaf morphology) and internal phenotypes (known defense-related phytohormones). These phenotypes were connected to single nucleotide polymorphism (SNPs) at the whole genome level to find out the underlying biological mechanisms. We confirmed that the derived populations have a higher number of genes under selection, including large suites of stress-related genes. Two of the derived populations (LA4117 and LA4330) belonging to the same genetic cluster were found to have completely opposite infection phenotypes, indicating that the adaptation to stress happens at the population level and not at the broader genetic cluster level. We show that resistance against a necrotrophic pathogen might be governed by both quantitative and qualitative mechanisms, as we found two SNPs in an R-gene (I2-like) associated with qualitative resistance and two SNPs in myc2-like transcription factor associated with quantitative resistance. Looking specifically at the derived populations, we found that different populations employ different phytohormones for susceptibility and resistance. Several hormone-associated SNPs were in the genes that are under selection in derived populations (Southern Coastal), such as 12 SNPs in the PFKA\_Ppi gene and 27 SNPs in the L-aspartate oxidase gene. Our study disentangled complex phenotypes of a wild species and showed how populations use different strategies to adapt to biotic stress when migrating to new habitats.

## **1-2 Exogenous dsRNA triggers sequence specific RNAi and fungal stress responses to control *Magnaporthe oryzae* in *Brachypodium distachyon***

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In vertebrates and plants, dsRNA plays crucial roles as PAMP and as a mediator of RNAi. How higher fungi respond to dsRNA is not known. We demonstrate that *Magnaporthe oryzae* (Mo), a globally significant crop pathogen, internalizes dsRNA across a broad size range of 21 to about 3000 bp. Incubation of fungal conidia with 10 ng/μL dsRNA, regardless of size or sequence, induced aberrant germ tube elongation, revealing a strong sequence-unspecific effect of dsRNA in this fungus. Accordingly, the synthetic dsRNA analogue poly(I:C) and dsRNA of various sizes and sequences elicited canonical fungal stress pathways, including nuclear accumulation of the stress marker mitogen-activated protein kinase Hog1p and production of ROS. Leaf application of dsRNA to the cereal model species *Brachypodium distachyon* suppressed the progression of leaf blast disease. Notably, the sequence-unspecific effect of dsRNA depends on higher doses, while pure sequence specific effects were observed at low concentrations of dsRNA (<0.03 ng/μL). The protective effects of dsRNA were further enhanced by maintaining a gap of at least seven days between dsRNA application and inoculation, and by stabilising the dsRNA in alginate-chitosan nanoparticles. Overall, our study opens up additional possibilities for the development and use of dsRNA pesticides in agriculture.

# 1-3 High Precision Quantification of small RNA Slicing Activity - Native Index Ligation-based Targeted Degradome Sequencing (NIL-TDS)

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RNA interference (RNAi) regulates gene expression through small RNA-guided cleavage of target mRNAs. This degradation activity is based on sequence complementarity of small RNAs and their targets. The application of small RNAs against mRNAs of pathogens provides a strategy with a high potential to control plant diseases. Designing small RNAs, however, requires the accurate evaluation of their degradome activities. Despite their potential significance in plant protection, existing degradome profiling methods (e.g. PARE, RLM-RACE) are labor-intensive, low in sensitivity, and lack quantitative precision. We therefore developed Native Index Ligation-based Targeted Degradome Sequencing (NIL-TDS), a novel, cost-effective Oxford Nanopore sequencing (ONT)-based method for direct, high-resolution detection and quantification of sRNA-mediated slicing events.

As a proof of concept, we used NIL-TDS on stressed plants, analysing the slice site of *Ath-miR400* and its target *PENTATRICOPEPTIDE REPEAT 1 (PPR1)* and generated, for the first time, quantitative data confirming the previously hypothesised reduction in slicing of PPR1. It indicated the high suitability of NIL-TDS for the accurate prediction and selection of small RNAs. It further approved NIL-TDS as a scalable standard to study RNAi-mediated regulation with unprecedented accuracy. This approach refines degradome analyses thereby streamlining the discovery and validation of naturally occurring silencing events in plant-microbe-interactions and facilitating the development of RNA-based crop protection strategies.

## 1-4 Processing and release of the phytoytokine Zip1

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*Zea mays* immune peptide 1 (Zip1) is a maize-specific phytoytokine involved in the salicylic acid (SA) signaling pathway. Zip1 is produced from its precursor, PROZIP1, through C- and N-terminal cleavage. Previously, two maize PLCPs, CP1 and CP2, were shown to cleave PROZIP1 in the apoplast. In turn, Zip1 activates PLCPs, induces the expression of pathogenesis-related (PR) genes, and leads to the accumulation of salicylic acid (SA). However, the mechanism of PROZIP1 processing, its subcellular localization, and the release of Zip1 are not yet fully understood. In this study, we investigated the localization of PROZIP1 and the steps involved in its processing to release Zip1. Our findings reveal that PROZIP1 is initially localized to the endoplasmic reticulum (ER) and the cytoplasm, where it undergoes processing. Our results indicate that the removal of the N-terminal PROZIP1 domain is essential for the translocation of the C-terminal part of PROZIP1 to the apoplast and that its release from the cell relies on exocytosis. We found that intracellular processing of PROZIP1 does not involve PLCP activity. Instead, we identified MC9, a calcium-dependent metacaspase, being responsible for the intracellular processing of PROZIP1 through arginine-dependent cleavage.

## **1-5 Genome-wide identification of LRR-RLPs highlights distinct patterns of presence and absence in defence and developmental-related clusters within and between tomato species.**

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Plants have evolved and diversified multiple receptor families to detect internal and external cues to adapt, survive and multiply. In this study, we developed an optimized pipeline for the genome-wide annotation of Leucine-rich repeats receptor-like proteins (LRR-RLPs) and divide them into complete (CRPs) and truncated (TRPs) LRR-RLPs. We assessed the diversity of CRPs in terms of copy number variation (CNV), chromosomal distribution, clustering in relation to their phylogenetic relationships and putative functions in defence or developmental activities. Next we use the pipeline to study patterns of presence, absence and expansion between four *Solanum spp.* (*S. lycopersicum*, *S. pimpinellifolium* (2093), *S. pennellii*, and *S. chilense*) and within three *S. pimpinellifolium* (2093, 1589, BGV066775).

We developed a standardised pipeline-“PlantLRR-PRRs” that does not require any manual curation and that is able to annotate genome-wide LRR-RLPs and differentiate them into CRPs and TRPs. CNV was observed in identified CRPs between 49 (*S. pimpinellifolium* (2093)) and 92 (*S. chilense*) across the species. Within the three tested *S. pimpinellifolium* accession, we found between 49 (LA2093) and 58 CRPs (LA1589). Next, we observed variation in the the chromosomal distribution of CRPs. *S. chilense* was also on top for having highest relative amount of CRPs located on most of the chromosomes (10 out of 12), whereas vice-versa for *S. pimpinellifolium* (LA2093). However, *S. pimpinellifolium* (LA2093) was noted for having the highest share of CRPs on chromosomes 2 and 4. Next, we used known CRPs from *Arabidopsis* and tomato as reference to assess phylogenetic relationships between the genome-wide annotated CRPs and assign putative roles in defence and development. The CRPs of *S. chilense* have largely expanded in the defence cluster, whereas for *S. pimpinellifolium* (LA2093), the expansion was observed in the developmental cluster, especially the CRPs found on chromosomes 2 and 4. We noticed a few orthologs of known developmental CRPs, including too-many-mouths (TMM) and the homolog of *A.thalaitiana* RLP29

were absent in *S. pennelli*, and RLP29 was absent in *S. pimpinellifolium* (LA2093), respectively as a result of a truncated signalling peptide in their protein sequences. Overall, we show that with a fully automated pipeline we can identify variation in CRPs in wild tomato species. Expanded families indicate novel opportunities to find new resistance-associated CRPs. Absence of known developmental CRP homologs offers avenues to study their roles in adaptation to new habitats.

## **1-6 Contrasting roles of D-clade ERFs in tomato defense against *Alternaria* pathogens**

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Ethylene is a pivotal phytohormone in plant immunity. Through RNA-seq analysis, we previously identified that ETHYLENE RESPONSE FACTORS (ERFs) within the D clade are instrumental in tomatoes for the early defense against the early blight disease complex (EBDC), which includes pathogens from the genus *Alternaria*. Transcriptional network analyses suggest ET-mediated JA, signalling to be the driver of this resistance. Interestingly, whereas clade D ERFs were specifically upregulated early after infection with *A. alternata*, infection with closely related *A. solani*, does not change the expression. Here we aim to elucidate the mechanisms underlying clade D ERF signalling and their possibly contrasting roles in combating the early blight pathogens. First we confirm the involvement of ethylene signaling by its precursor, 1-aminocyclopropane-1-carboxylate (ACC), and an inhibitor, aminoethoxyvinylglycine (AVG), to assess its impact on resistance to *Alternaria*. We further investigated the functional roles of three D clade ERF genes using virus-induced gene silencing (VIGS) and transient overexpression (OE) of ERFs. This reveals that gene-silenced plants exhibit enhanced susceptibility to *A. alternata*. Conversely, overexpression of ERFs in tomatoes increased susceptibility to *A. solani*. To delve deeper into the regulatory mechanisms of ERFs on downstream genes, we conducted qPCR analyses to examine the modulation of defense responses, including jasmonic acid (JA) and pathogen-related (PR) genes in OE and silenced plants, and found that ERFs play contrasting roles in the regulation of JA signalling against both *Alternaria* species. Our results affirm the critical role of D-clade ERFs in defending against *Alternaria alternata*. At the same time, they suggest that closely related species can modulate, utilize, or suppress these responses to their advantage. This provides new insights into the importance of fine-tuning homeostasis and balancing ET-mediated defence signalling against necrotrophic pathogens, shedding light on the molecular basis of plant-pathogen interactions.

## **1-7 Uncovering cell-specific responses of barley cells in response to *Magnaporthe oryzae* infection**

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Upon pathogen attack, plants rely on the defense mechanisms of individual cells. To overcome resistance, pathogens secrete specific molecules, known as effectors, which help them evade, suppress, or manipulate the immune response of each penetrated cell. However, even if a pathogen manages to suppress the immune response in one cell, neighboring cells may be able to resist invasion. To gain a comprehensive understanding of the genetic and metabolic differences between susceptible and resistant plant cells, as well as the mechanisms by which pathogens manipulate the host immune system, it is not sufficient to analyze host-pathogen interactions at the whole tissue level, instead investigations at the single-cell level are mandatory. Consequently, we aimed to develop a strategy to selectively target barley cells colonized by the fungus *M. oryzae* and investigate their transcriptomic profiles in comparison to those of uninfected cells. Therefore, we first generated protoplasts from infected barley leaves, then the RNAs from each cell were barcoded, followed by RNA sequencing. Cells with similar gene expression profiles were clustered into distinct groups. Subsequently, these clusters were analyzed for marker genes associated with infection using the MapMan tool. Through this analysis, we aim to identify clusters that contain infected cells and thereby pinpoint barley genes that play crucial roles in immune response.

# 1-8 Interplay of the fungal endophyte *Fusarium verticillioides* with the pathogenic smut fungi *Ustilago maydis* and *Sporisorium reilianum* in maize

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The smut fungi *Ustilago maydis* and *Sporisorium reilianum* are major maize pathogens that cause significant yield losses. *U. maydis* infects aerial parts and induces tumor formation, while *S. reilianum* infects roots, spreads systemically, and alters flowering.

The soil borne ascomycete *Fusarium verticillioides* co-evolved with maize and is widely prevalent as an endophyte. A growing number of studies are exploring the role of *F. verticillioides* endophytes in protecting their host plants as antagonists of pests and pathogens. Most notably, Lee et al. (2009) found that endophytic strains of *F. verticillioides* reduces disease severity of *U. maydis* in maize.

We aim to understand how maize modulates its microbiome to mitigate stress from *U. maydis* and *S. reilianum*, potentially by recruiting beneficial bacteria. We also seek to investigate the molecular mechanisms behind *F. verticillioides* antagonism against *U. maydis* and explore possible interactions with the closely related smut, *S. reilianum*. To achieve these goals, we will isolate endophytes and epiphytes from the phyllosphere of maize infected with *U. maydis* and *S. reilianum*. The bacterial community will be compared to uninfected plants to infer potential interactions. Fungal-fungal interactions will be assessed through co-inoculations of various *F. verticillioides* strains with *U. maydis* and *S. reilianum* in multiple maize lines. RNA sequencing of significant interactions will help identify and characterize genes involved in *F. verticillioides* antagonism.

Ultimately, understanding microbial antagonism and the genes mediating these interactions is key to developing novel strategies for managing plant fungal pathogens.

## Reference

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# 1-9 Functional adaptation of a transcriptional activator effector family in smut fungi and beyond

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Phytopathogens secrete effectors that inhibit the plant immunity and modulate the host's transcriptome to facilitate their infection. In our previous study, we functionally characterized Sts2, a novel transcription activator effector in *Ustilago maydis*1. Sts2 activates the expression of maize leaf developmental regulators to induce hyperplastic tumor formation. Interestingly, Sts2 orthologs underwent functional diversification between smut fungi *U. maydis* and *Sporisorium reilianum*2. However, the mechanism of this neo-functionalization remains to be elucidated. Here, we computed the ancestral Sts2 sequence based on all available orthologs in smut fungi. We tested ancestral Sts2 and present orthologs for their activities as transcriptional activator, as well as their virulence function in *U. maydis*. Our results suggested that Sts2 orthologs are a functional transactivator effector family in smut fungi, and neo-functionalization occurred during the speciation of *U. maydis* to modulate UmSts2 for hyperplastic tumor induction. In addition, AlphaFold based 3D structure analysis identified two structurally variable domains in Sts2. Expression of chimeric Sts2 versions experimentally validated that these variable domains are responsible for the functional difference between UmSts2 and SrSts2. Furthermore, we identified new transactivation effectors (TAEs) from *U. maydis* and the fungal pathogens *Magnaporthe oryzae* and *Bipolaris maydis*, demonstrating the presence of TAEs in different phytopathogens. References: 1. Zuo, W., Depotter, J. R. L., Stolze, S. C., Nakagami, H. & Doehlemann, G. A transcriptional activator effector of *Ustilago maydis* regulates hyperplasia in maize during pathogen-induced tumor formation. *Nat Commun* 14, 6722 (2023). 2. Zuo, W., Depotter, J. R. L., Gupta, D. K., Thines, M. & Doehlemann, G. Cross-species analysis between the maize smut fungi *Ustilago maydis* and *Sporisorium reilianum* highlights the role of transcriptional change of effector orthologs for virulence and disease. *New Phytologist* 232, 719–733 (2021).

# **1-10 A fight for survival: The role of the NADPH oxidase RbohD in the *Botrytis cinerea* - plant interaction**

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## **2-1 *Bacillus halotolerans* B21 Effective Against *Fusarium graminearum* and *Erwinia amylovora* - Potential use as a Biocontrol Agent**

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## 2-2 Identification of AVRA effector targets in barley

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*Blumeria hordei* (Bh) causes the powdery mildew disease of barley. The RNase-like Bh AVRA effectors are recognized by immune receptors encoded at Mildew locus a (Mla) in resistant barley lines. The maintenance of AVRA 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 investigate the virulence function of AVRA effector, we aim to identify their host targets inside barley cells. To achieve this, we applied a combination of in vivo proximity-dependent protein labelling and co-immunoprecipitation using AVRA-expressing stable transgenic barley lines. We identified multiple candidate interactors of AVRA1, AVRA7, AVRA9, AVRA10 and AVRA13. Our data suggests that AVRA13 but not the other AVRA effectors specifically targets the barley Strubbelig Receptor Family3 (SRF3) receptor-like kinase to modulate host immunity. AVRA13 can interact with the cytoplasmic domains of only certain barley SRF3 isoforms and influences SRF3 plasma membrane depletion. Together our data suggests that a) the intrinsic functions of individual Bh AVRA effectors differ despite the fact that the effectors are structurally related and b) that SRF3 may modulate immunity towards powdery mildew and potentially other pathogens.

## 2-3 Network inference reveals distinct transcriptional regulation patterns in barley

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Barley is an important agricultural crop in Germany, but its yield faces threats from abiotic stresses like drought and biotic stresses such as Fusarium head blight (FHB). FHB is caused by a complex of Fusarium species, with *Fusarium culmorum* (*Fc*) regularly found on barley. FHB infection can lead to significant grain losses and infected grains can pose risks to human and livestock health. To enhance crop resilience, it is crucial to understand the molecular mechanisms that contribute to barley's susceptibility and potential resistance to these stresses.

In this study, we aimed to investigate the gene regulatory interactions in barley under the combined stress of drought and FHB<sup>1</sup>. We utilized a gene regulatory network (GRN) approach<sup>2</sup> to analyze gene expression in barley, identifying key regulators that influence gene expression during both infection and drought stress adaptation. To enhance our understanding of the expression data, we also performed a weighted gene correlation network analysis (WGCNA)<sup>3</sup> and associated the identified modules with external traits, such as measured phytohormones and the quantified amount of fungal DNA.

We compared the results from the tree-based GENIE3 algorithm, which infers regulatory interactions, with the co-expression-based WGCNA approach. Despite their different mathematical frameworks, both methods produced highly similar gene modules, supporting the robustness of our findings. The Jaccard Distance is a metric used to measure the similarity between different groups. By applying this metric to genes and their associated regulators, we identified key transcription factors that are strongly linked to responses to either infection or drought. Using published data, we were able to verify some of our predictions with validated, peer-reviewed research. Together, these two techniques serve as powerful tools for identifying novel regulators and their targets. This enhanced understanding is crucial for elucidating the roles of these regulators in various biological phenomena or diseases.

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## **2-4 Characterisation of conserved virulence factors of *Puccinia sorghi* and *Ustilago maydis***

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To successfully colonise their hosts, plant pathogens rely on virulence factors. Virulence factors include secreted effector proteins that target molecules, pathways and structures of the host plant, as well as cellular structures, molecules, and regulatory systems that enable the pathogen to infect its host, without directly targeting the host. So far many virulence factors of biotrophic pathogens have been identified and functionally characterized. However, in obligate biotrophic pathogens, studying virulence factors remains challenging due to the lack of efficient genetic transformation and gene deletion techniques. In this study, we aim to analyse virulence factors of the obligate maize pathogen *Puccinia sorghi* in its native host plant, by using the genetically tractable smut fungus *Ustilago maydis* - maize pathosystem. A pool of putative *U. maydis* virulence factors, upregulated upon infection and predicted to be secreted, was screened for homologues in *P. sorghi*. This approach yielded 40 putative virulence factors with homologues in both pathogens. Deletion of seven virulence factors resulted in reduced virulence of *U. maydis*. So far, the virulence of two deletion mutants could partially be restored by the introduction of the respective *P. sorghi* homologue, suggesting that these virulence factors are functionally conserved between rust and smut fungi. In the next steps, we will assess if the remaining *P. sorghi* homologues can restore *U. maydis* virulence, as well as functionally characterize selected virulence factors.

## **2-5 The host-specificity factor SOVIG9 of *S. reilianum* affects phytoalexin induction in *Sorghum bicolor***

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*Sporisorium reilianum* is the causal agent of head smut in sorghum and maize. It exists in two host-specific formae speciales, *Sporisorium reilianum* f. sp. *reilianum* (SRS) and *Sporisorium reilianum* f. sp. *zeari* (SRZ) that are pathogenic on sorghum and maize, respectively. While SRS-infected sorghum shows almost no visible symptoms in early infection stages, sorghum inoculation with SRZ induces heavy defence responses including the generation of reddish-brown phytoalexins (PA). We identified an effector protein of SRS, SOVIG9, that suppresses the PA response of sorghum. Transcriptome comparison of wildtype- and SRS $\Delta$ SOVIG9 deletion strain-inoculated sorghum leaves showed upregulation of defence-related sorghum genes, including PA biosynthesis and receptor-like kinase genes. Currently, we identify functional domains of the effector protein as well as plant protein interactors to elucidate the molecular function of SRS-SOVIG9 in the suppression of the sorghum PA response.

In SRZ, a second shortened splice-variant of SOVIG9 is expressed. When this short SRZ splice variant is used to complement SRS $\Delta$ SOVIG9 deletion strains, the fungus induces a strong PA response similar to complementation strains carrying non-functional, C-terminally tagged versions of the SRS effector that are recognized as avirulence proteins in sorghum. Therefore, the PA response-suppressing effector SOVIG9 seems to have been subject to forma specialis-specific alternative splicing, generating an effector version in SRZ that is recognized by the sorghum defense proteins and contributes to the inability of SRZ to cause disease on sorghum.

## 2-6 Deciphering the Molecular Mechanism of a Fungal Effector Protein that Impairs Plant Development

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Biotrophic pathogens employ various strategies to survive and propagate within host plants, yet the mechanisms behind these processes remain poorly understood. One such biotrophic pathogen is *Sporisorium reilianum* f.sp. *zetae*, which causes head smut disease in *Zea mays*. Using comparative genomics and deletion studies a fungal effector protein (SUPPRESSER OF APICAL DOMINANCE 1) was identified to be responsible for the formation of sub-apical ears, a typical symptom observed upon infection. Using yeast 2 hybrid screening, E3 ubiquitin ligase *ZmRGLG1* was found to be one of the strongest interaction partners. In *Arabidopsis thaliana* the homologs, *AtRGLG1* and *AtRGLG2* are known to have a role in the abiotic stress response by controlling the stability of transcription factor ERF53, and kinases MAPKKK18. Among the 5 RGLGs, Yeast 2 hybrid analysis suggested the strongest interaction of SAD1 with *AtRGLG2*, which is a homolog of *ZmRGLG1*. We hypothesise SAD1 is interfering with the natural function of *AtRGLG2* leading to an accumulated stress response resulting in a loss of apical dominance via a stress-induced morphogenic response. *In vitro* ubiquitination assays showed SAD1 can be ubiquitinated in the presence of both *ZmRGLG1* and *AtRGLG2*. The possibility of SAD1 being a preferred substrate for RGLG2 over their natural targets would be analysed using TriFC, Yeast-three-hybrid assays, ROS burst assays and drought stress assays. Further, we want to prove the ubiquitination of SAD1 by RGLG proteins in planta using IP-LC/MS. This will provide insights into how effector-induced phenotypic changes in plants are utilized by biotrophic pathogens as a strategy for survival and propagation.

## **2-7 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 29x *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 29x 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. The search for remaining CDIPs is ongoing, to generate finally a non-necrotrophic *B. cinerea* mutant. Based on their homology to known CDIPs, we have identified three new CDIPs that are currently under investigation. 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 the functional redundancy of fungal virulence factors.

## 2-8 Exploring potential benefits of biostimulant treatments in lupin cultivation

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Lupin is a valuable alternative protein source for both human food and animal feed. White- and narrow-leafed lupins are cultivated in Germany alongside traditional protein crops such as broad bean and pea and represent an attractive alternative to (GMO-) soybean from national- and international markets. Our project “P<sup>3</sup>roLucas” (Optimization of plant performance and products for lupin cascade use) aims at promoting and improving lupin cultivation in Germany and encompasses a wide range of research topics ranging from exploration of lupin alkaloids for industrial use to sequencing of the Andean lupin genome as a resource for future breeding programmes [1]. Central topic is the smart use of so called biostimulants as (partial) alternatives to traditional plant protection products which are increasingly being phased out under European and national regulatory policies. Therefore, we are exploring effects of commercially available biostimulants in narrow-leafed lupin (*Lupinus angustifolius*) on plant growth and protection against biotic- and abiotic stresses. Products based on *Bacillus spec.* were identified as the most promising candidates. We implemented a combined approach of seed treatments including the so-called seed-priming strategy, which is close to agricultural practice. In addition, the localisation of bacteria after seed treatments and their influence on plant development and induction of resistance to lupin anthracnose, were investigated. We present results from lab-scale experiments, currently being validated in field experiments and analyses at the metabolic- and transcriptomic level, using consolidated and newly generated genomic resources within this project.

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## 2-9 Interbacterial competition by R-type phage tail-like particles

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Bacteria use various mechanisms to outcompete other microorganisms in their habitat. One mechanism, that is particularly specific against closely related bacteria, is the biosynthesis of capsid-free Contractile Phage Tail-like Particles (CPTPs). These structures are evolutionarily related to phages but lack a capsid. They bind specifically to the surface structures of the target cells via special recognition features, e.g. through ‘tail fibers’. CPTPs can function in different ways, either by piercing the cell membrane and disrupting the osmotic balance (Tailocins), or by transferring effectors or toxins (eCIS). *Kosakonia radicincitans* DSM 16656T is a bacterium isolated from winter wheat. When used as an inoculum, the strain promotes the growth of a variety of agricultural relevant crops. *K. radicincitans* DSM16656T expresses a tailocin gene cluster, which contains three different genes for tail fiber proteins that differ significantly from closely related strains in particular sequence regions. Tailocins produced by *K. radicincitans* DSM16656T could therefore have a broad target spectrum. To investigate how *K. radicincitans* Tailocin production potentially influences plant-microbe interactions, we started to explore the target spectrum of purified Tailocins against plant endophytic and plant pathogenic bacterial strains.

## **2-10 Application of beneficial bacteria as an induced resistance-based approach for enhanced resilience of spring barley against *Ramularia collo-cygni* infection**

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One of the most important fungal phytopathogens in barley cultivation (*Hordeum vulgare*) today is *Ramularia collo-cygni*. The hemibiotrophic fungus is associated with the disease Ramularia leaf spot and is held responsible for significant yield and grain quality losses. The susceptibility of barley varieties to *R. collo-cygni* and the increasing resistance of the pathogen to common fungicides underline the need to better understand this pathogen and to look for alternative plant protection strategies.

Enhanced plant resilience to a broad spectrum of diseases could mitigate the problems mentioned above. Among other things, it is known that contact between the host plant and beneficial rhizobacteria stimulates induced systemic resistance (ISR), which enables the plant to respond faster and more strongly to a challenge from pathogens and pests. In PrimedPlant-2, *Bacillus spp.* inoculation to barley plants indicated a positive effect on resistance to natural infection with *R. collo-cygni* in the field. To evaluate the potential impact of *Bacillus* inoculation in the symptomatic and asymptomatic growth phases of the pathogen, we are currently developing a methodological toolbox: we established a reliable production of fungal conidiospores in axenic culture that allows inoculum production for infection experiments. The first infections were successfully carried out on detached spring barley leaves. Confocal laser scanning microscopy allows observation of the colonization in the leaf tissue using fungal cell wall-specific staining. We are currently establishing a spore transformation system to enable genetic manipulation of the fungus (e.g. eGFP tagging). The conditions for whole plant infection and symptom development will be evaluated, followed by greenhouse trials involving treatment with *Bacillus spp.* and assessment of the influence of altered abiotic conditions such as light and drought stress. To investigate the effects of *Bacillus* inoculation on natural infestation with *R. collo-cygni*, infection progress will be monitored by species-specific qPCR in field trials, including eight genetically different spring barley lines.

## 2-11 Avirulence depletion assay: Combining *R* gene-mediated selection with bulk sequencing for rapid avirulence gene identification

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Wheat production faces significant threats from various fungal pathogens. Resistance breeding in wheat often depends on the utilization of resistance (*R*) genes that encode diverse immune receptors capable of detecting specific avirulence (*AVR*) effectors, thereby triggering an immune response. While the cloning of *R* genes has advanced in recent years, the identification of *AVR* genes in many fungal pathogens, including *Blumeria graminis* f.sp. *tritici* (*Bgt*), lagged behind. As a result, the strategic deployment of these resistance sources, informed by pathogen diversity studies, remains underexplored. To address this issue, we developed an new “avirulence depletion (*AD*) assay” designed for the rapid identification of *AVR* genes in *Bgt*. This assay involves selecting a segregating haploid F1 progeny population on a resistant host, followed by bulk sequencing, thereby allowing for rapid identification of avirulence candidate genes with high mapping resolution. The *AD* assay represents a powerful and cost-effective tool for the rapid identification of *AVR* genes in *Bgt*, with the potential to enhance pathogen-informed breeding strategies for employing novel *R* genes and regionally tailored gene deployment against *Bgt* and other pathogens.

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