Matthias Hahn (Hrsg.)

58. Jahrestagung des DPG-Arbeitskreises Mykologie

42. Jahrestagung des DPG-Arbeitskreises Wirt-Parasit-Beziehungen 2023

Zusammenfassungen der Arbeitskreisbeiträge

16./17. März 2023

an der TU München in Freising-Weihenstephan

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Arbeitskreise ‚Mykologie' und ‚Wirt-Parasit-Beziehungen' 2023

Die gemeinsame Tagung der Arbeitskreise ‚Mykologie' und ‚Wirt-Parasit-Beziehungen' fand am 16./17. März 2023 an der TU München in Freising-Weihenstephan statt.

Die nächste Tagung ist für den 29.2/1.3. oder 7.3/8.3.2024 an der Universität Bonn geplant.

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

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

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

Tagung der beiden Arbeitskreise Wirt-Parasit-Beziehungen und Mykologie der Deutschen Phytomedizinischen Gesellschaft (DPG), am 16. und 17. März 2023.

Nachdem aufgrund der Corona-Pandemie die Jahrestagung der Arbeitskreise 2020 ausfallen musste und in beiden folgenden Jahren nur in Form einer digitale Videokonferenz stattfinden konnte, war es nun endlich wieder möglich, alte und neue Kolleginnen und Kollegen in Präsenz bei der Tagung der Arbeitskreise »Wirt-Parasit-Beziehungen« und »Mykologie« zu treffen und sich persönlich und wissenschaftlich auszutauschen. Das Treffen fand bei sonnigem Wetter auf dem Campus Weihenstephan der TU München in Freising statt und wurde von unserem Gastgeber und 3. Vorsitzenden der DPG, Prof. Ralph Hueckelhoven, und seinem Mitarbeiterteam perfekt organisiert. Es waren 80 Teilnehmende vor Ort, die ihre aktuellen Forschungsergebnisse in 28 Vorträgen und 22 Postern präsentierten. In zwei Poster-Pitch-Sessions wurden die Poster in 2-minütigen Kurzpräsentationen vorgestellt. Dieses Format fand einen sehr guten Anklang bei allen Teilnehmenden und führte zu lebhaften Diskussionen bei den anschließenden Postersessionen. Die Möglichkeit der Co-Moderation der Sektionen durch den Nachwuchs wurde wieder gerne angenommen.

Die Thematik der diesjährigen Beiträge war ausgesprochen breit, sowohl bzgl. der Vielfalt an untersuchten Pathosystemen als auch in Hinblick auf die Fragestellungen und experimentellen Strategien. Generell war der Trend sichtbar, dass die phytopathologische Grundlagenforschung zunehmend in die Nähe einer möglichen Anwendung der erzielten Erkenntnisse vordringt. Dies wurde deutlich bei Beiträgen zum Verständnis der Rolle des Klimas bei der Entstehung von Pflanzenkrankheiten, dem Einsatz von kleinen RNAs und nützlichen Mikroorganismen als Alternativen zu chemischen Behandlungsmethoden, dem zunehmenden Verständnis der komplexen molekularen Mechanismen von Krankheitsentstehung und Pflanzenabwehr, und dem Einsatz von Multispektral-sensoren und Drohnen zur präzisen Diagnose der Krankheitsentwicklung im Feld.

Prof. Matthias Hahn (RPTU Kaiserslautern) gab nach fünfjähriger Amtszeit die Leitung des Arbeitskreises ‚Wirt-Parasit-Beziehungen' ab. Sein Stellvertreter, Prof. Ulrich Schaffrath (RWTH Aachen) und Prof. Hueckelhoven dankten ihm im Namen aller Teilnehmenden für seinen Einsatz. Anschließend wurde Prof. Gunther Döhlemann (Universität Köln) einstimmig als Nachfolger gewählt. Wir sind überzeugt davon, dass er gemeinsam mit Prof. Schaffrath den größten Arbeitskreis innerhalb der DPG erfolgreich in die Zukunft führen wird.

Prof. Armin Djamei (Universität Bonn) hat sich erfreulicherweise dazu bereit erklärt, die nächste Tagung der beiden Arbeitskreise auszurichten, sie wird am 29. Februar/ 1. März, oder am 7./ 8. März 2024 stattfinden.

Prof. Dr. Matthias Hahn
Prof. Dr. Gunther Döhlemann
Dr. Monika Heupel

Deutsche Phytomedizinische Gesellschaft e.V.

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12. Februar 2023

Jahrestagung der DPG-Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“ am 16./17. März 2023 an der TU München in Freising

Sehr geehrte Damen und Herren, Liebe Kolleginnen und Kollegen,

in diesem Dokument finden Sie das Programm der Beiträge zur gemeinsamen Tagung DPG-Arbeitskreise ‚Mykologie‘ und ‚Wirt-Parasit-Beziehungen‘ am 16./17. März 2023.

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

Das Zeitfenster für einen Vortrag ist wie bisher 15 min (10-12 min Redezeit, 3-5 min Diskussion). Wir bitten Sie, die Zeit einzuhalten und die Themen der Vorträge gut einzuführen, damit auch Nichtfachleute davon profitieren. Da sich die Poster Pitch Sessions in den vergangenen Online-Tagungen bewährt haben, wollen wir sie erstmals auch in einer Präsenztagung durchführen. Dabei stellen die AutorInnen ihre Poster zunächst in Form einer maximal 2-minütigen Kurzpräsentation vor. Wir bitten hierzu um Vorab-Zusendung einer aus 1-3 Folien bestehenden Zusammenfassung der Highlights Ihres Posters als pdf- oder ppt-Dokument bis zum 14. März, an hahn@biologie.uni-kl.de.

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

Mit den besten Grüßen

Matthias Hahn und Marco Thines

PROGRAMM

Donnerstag, 16.3.2023

13:00 Uhr BEGRÜSSUNG

VORTRAGS-SESSION 1 (Leitung: Gunther Döhlemann)

13:10 Uhr Christian Schwarz (Univ. Regensburg): Susceptibility of European maize lines to *Ustilago maydis* infections in a changing climate

13:25 Uhr Armin Djamei (Univ. Bonn): Lessons to learn from a gall-inducing fungus

13:40 Uhr Julian Maroschek (TU München): A simple method for the screening of receptor-ligand interactions using in planta expressed ectodomains

13:55 Uhr Florencia Casanova (RWTH Aachen): Application of an inducible promoter for analysis of virulence factors during *Magnaporthe oryzae* infection in planta

14:10 Uhr Ena Šečić (Univ. Gießen): Non-coding RNAs as mediators of beneficial plant symbioses

14:25 Uhr Marion Müller (TU München): Ancient variation in avirulence effectors underlies the rapid resistance breakdown of two introgressed rye resistance genes in wheat

14:40 – 15:05 Uhr POSTER PITCH SESSION 1 (2 min Kurzpräsentationen)

1-2: Alex Wegner (RWTH Aachen): The effector MoNudix is required for full virulence of *Magnaporthe oryzae* on barley

1-3: Kishore Ramesh Kumar (Univ. Jena): Protein pull-down and proteomic analysis of the SAD1 smut effector protein and its interacting proteins from *Arabidopsis thaliana* and *Zea mays* for improved cob productivity in maize

1-4: Alexandra Schmidt (Univ. Hohenheim): Establishing RNA spray-based solutions for sustainable control of the biotrophic fungal pathogens *Phakopsora pachyrhizi* and *Blumeria graminis* f.sp. *hordei*

1-6: Louisa Wirtz (RWTH): Flow cytometric purification of *Magnaporthe oryzae* infected barley cells for transcriptome analysis

1-7: Silvana Laupheimer (TU München): Volatile-mediated signaling in barley induces metabolic reprogramming and resistance against the biotrophic fungus *Blumeria hordei*

1-8: Christian Trautmann (Univ. Hohenheim): A UAV based Monitoring System for the early Detection of Plant Diseases in Field Vegetable Cultures

1-9: Man Mu (Univ. Frankfurt/ Senckenberg) Diversity of *Peronospora* causing downy mildew disease on *Myosotis* and *Veronica*

1-11: Behnous Hosseini (Univ. Hohenheim): Detection of the soybean pathogen *Diaporthe* spp. in soil

1-12: Carolin Popp (JKI Dossenheim): Sea buckthorn dieback in Northern Germany: results of fungal community sequencing

1-13: Claire Maria Haumann (TU München): Inhibition of *Alternaria solani* and *Phytophthora infestans* through volatile organic compounds emitted by *Trichoderma* spp.

15:05 – 15:45 Uhr KAFFEEPAUSE & POSTER SESSION 1

VORTRAGS-SESSION 2 (Leitung: Matthias Hahn & Daniela Nordzieke)

15:45 Uhr Maurice König (Univ. Köln): Maize phytocytokines and microbial-patterns trigger antagonistic features in co-incidence with wounding and fungal pathogens

16:00 Uhr Alejandra Vielba Fernandez (RPTU Kaiserslautern): Addressing redundant roles of phytotoxic proteins of *B. cinerea* for necrotrophic infection by multi-k.o. mutagenesis

16:15 Uhr Tobias Müller (RPTU Kaiserslautern): Licence to kill: *Botrytis* Hip1 alters plant immunity to defeat its hosts

16:30 Uhr Daniela Nordzieke (Univ. Göttingen): Hyphal fusions enable efficient nutrient distribution in *Colletotrichum graminicola* conidiation and symptom development on maize

16:45 Uhr Janina Werner (Univ. Köln): Molecular analysis of a fungal hybrid of *U. maydis* and *S. reilianum*

17:00 Uhr Martin Herde (Univ. Jena): The histone lysine methyltransferase Ash1 of *Sporisorium reilianum* negatively regulates effector gene expression in axenic culture

17:15 Uhr Matthias Kretschmer (Univ. Vancouver, Canada): The monothiol glutaredoxin Grx4 is involved in iron sensing, secondary metabolism, fungal cell surface functions and virulence in *Ustilago maydis*

17:30 Uhr Christoph Bartram (TU München): Potential function of anionic phospholipids in HvRACB-supported susceptibility to *Blumeria hordei*

17:45 Uhr Bilal Ökmen (Univ. Tübingen): A conserved enzyme of smut fungi facilitates cell-to-cell extension in the plant bundle sheath

ca. 19 Uhr Abendessen im Bräustüberl Weihenstephan (Weihenstephaner Berg 10, 85354 Freising)

Freitag, 17.3.23

VORTRAGS-SESSION 3 (Leitung: Monika Heupel & Carolina Schroeder)

8:30 Uhr Martin Rieker (Univ. Hohenheim): Efficacy of new fungal and bacterial BCAs against Fusarium Head Blight in wheat for NOcsPS cropping systems

8:45 Uhr Bennet Rohan Devasahayam (Univ. Halle): Microbial Biological Control Agents (MBCAs): Consumers' friends or foes?

9:00 Uhr Lala Aliyeva-Schnorr (Univ. Halle): Urease inhibitors for soil microorganisms reduce severe disease symptoms of fungal pathogens on crop leaves

9:15 Uhr Carolina Schroeder (BASF Limburgerhof): Adaptation of the sensitivity of *Alternaria solani* towards different fungicides

9:30 Uhr Facundo Ispizua Yamati (IFZ Göttingen): Combining data-driven deep learning model and environmental epidemiology knowledge for prediction *Cercospora* leaf spot in sugar beet using multispectral sensors and weather stations

9:45 Uhr Abel Barreto Alcantara (IFZ Göttingen): Leaf analysis for quantitative resistance and disease control using multispectral UAV images: case of *Cercospora* leaf spot in sugar beet

10:00 – 10:40 Uhr POSTER PITCH SESSION 2 (2 min Kurzpräsentationen)

2-1: Henriette Leicher (TU München): A powdery mildew fungus hijacks endogenous FER-RALF signaling to establish successful host colonization

2-2: Lina Munoz (TU München): Comparative metabolomics of *Solanum lycopersicum* to elucidate anti-fungal defence mechanisms

2-3: Marco Loehrer (RWTH Aachen): Cascade use of lupin plants: protein for food and feed and source for high-value chemicals

2-4: Nassim Safari (RPTU Kaiserslautern): Adressing redundant roles of phytotoxic proteins for necrotrophic infection of Botrytis cinerea by multi-k.o. mutagenesis

2-5: Yoon Joo Lee (Univ. Köln): The Pec effector complex of *Ustilago maydis* interferes with carbohydrate metabolism in maize

2-6: Jennifer Thielmann (Univ. Gießen): Wheat diversity set analyses reveal genotype-specific priming capacities induced by beneficial endophytes

2-8: David Scheuring (RPTU Kaiserslautern): Deciphering host plant penetration of the pathogenic fungus *Botrytis*

2-9: Justus Detring (IFZ Göttingen): Proof of concept for spectral phenotyping of Syndrome Basses Richesses in sugar beet using hyperspectral sensors and machine learning

2-10: Zarah Sorger (Univ. Köln): Mechanisms of basidiomycete yeast function in complex leaf microbial communities

2-11: Yixuan Yang (IFZ Göttingen): Impact of cultivar resistance on *Cercospora beticola* epidemiology on sugar beet

2-12: Saeed Muhammad (RPTU Kaiserslautern): Searching powdery mildew-resistant oaks

2-13: Johana Misas Villamil (Univ. Köln): Maize phytocytokines modulate pro-survival host responses and pathogen resistance

10:40 – 11:25 Uhr KAFFEEPAUSE & POSTER SESSION 2

11:25 Uhr Wahl des neuen Leiters und Stellvertreters des Arbeitskreises ‚Wirt-Parasit-Beziehungen'

VORTRAGS-SESSION 4 (Leitung: Ulrich Schaffrath & Marco Löhrer)

11:35 Uhr Martin Stegmann (TU München): Coordination of cell surface immunity and N limitation by CEP-mediated signalling

11:50 Uhr Lukas Rollwage (IFZ Göttingen): VPg carrying members of the virus yellows diseases complex interact with different translation initiation factors of sugar beet

12:05 Uhr Seema Pawar (Univ. Hohenheim): Assessment of the defense stimulus induced by UV-C light in *Agrostis stolonifera*

12:20 Uhr Isabel Saur (Univ. Köln): An avirulence effector from barley powdery mildew fungus can supress Mildew locus A resistance

12:35 Uhr Bernhard Werner (Univ. Gießen): Analysis of codon usage and allele frequencies reveal the double-edged nature of cross-kingdom RNAi

12:55 Uhr Maria Ladera Carmona (Univ. Gießen): Non-coding RNAs as smart bio-fungicides in future crop protection

13:10 Uhr Stephen Knobloch (Univ. Frankfurt/ Senckenberg): AGRIFUTURE: a tool for rapid plant pathogen detection using mobile nanopore sequencing

13:20 Uhr Ort und Termin der nächsten Arbeitskreistagung 2024; Verabschiedung

Jahrestagung der DPG-Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“, 16./17. März 2023 an der TU München in Freising

ABSTRACTS

VORTRAGS-SESSION 1

V1-1: Susceptibility of European maize lines to *Ustilago maydis* infections in a changing climate

Schwarz C, van der Linde K

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With an average annual production of 1.1 billion tons worldwide, maize is one of the most important staple crops. However, increasingly stronger effects of climate change pose a threat to its productivity. Rising temperatures are not only predicted to directly reduce yields but also indirectly by facilitating increased infections with plant pathogens. To test this prediction, we investigated the influence of different temperatures on infections of maize (*Zea mays*) with the smut fungus *Ustilago maydis*. We infected 17 different European maize lines at different temperatures using modelled field temperatures of 1985, 2050, or a short heat wave of three days. Disease ratings were performed, which revealed a strong effect on susceptibility under predicted temperatures as well as cultivar-specific effects. Next, a RNAseq analysis will be performed to identify temperature- and cultivar-specific susceptibility factors, and the resulting data will be used to establish a novel, GMO-free technique for plant protection. The latest results from these experiments will be presented.

V1-2: Lessons to learn from a gall-inducing fungus

Armin Djamei (Univ. Bonn)

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Smut fungi form a large group among the basidiomycetes and are biotrophic specialists in infecting a diverse set of mainly grasses, among them important crops like sorghum, millet, barley and maize. The maize smut fungus *Ustilago maydis* serves as an important model for smuts fungi and induces prominent galls on all aerial parts of its host, reflecting a metabolic and developmental reprogramming of the plant. This massive manipulation of the host is achieved with the help of fungal secreted molecules, so called effectors. In a systematic approach we screened in the past decade hundreds of putative effector proteins to identify their specific place of action and their functions on the plant side. Here I will present our current molecular understanding of the fungal effectome and the biotrophic interaction between the fungus and its host plant maize. Main focus will be given to a group of effectors suppressing the central negative regulator Topless in plants, thereby explaining various central aspects of biotrophy.

V1-3: A simple method for the screening of receptor-ligand interactions using *in planta* expressed ectodomains

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In plants, the interaction between membrane-localized receptors and their cognate ligands initiates and tightly controls various physiological processes. Research efforts in recent years have uncovered many such receptor-ligand pairs and the methodological toolbox to study their interactions is continuously expanding. However, such elaborate techniques usually require knowledge about the identity of both receptor and corresponding ligand.

We recently enriched an elicitor-active fraction from different *Fusarium* species and related fungi, which induces pattern-triggered immunity responses in a wide range of plant species. In the model plant *Arabidopsis thaliana* those responses are fully dependent on the leucine-rich repeat receptor-like kinase (LRR-RLK) MIK2 (Coleman *et al.* 2021). Nevertheless, the identity of the elicitor-active molecule remains elusive and a direct role of MIK2 in its perception is still to be confirmed. Consequently, we sought to develop a suitable method to demonstrate specific interaction between MIK2 and the elicitor-active molecule.

For this purpose, we have adopted the depletion-binding assay developed by Shu and colleagues (2021) to fit our needs. We expressed affinity-tagged ectodomains of various LRR-RLKs *in planta*, collected them with apoplastic wash fluids and immobilized the ectodomains on matching affinity-columns. The ligands of interest were then subjected to the ectodomain-coated columns, and the flow-through was tested for remaining or depleted activity with a biological assay for elicitor responses. Using this method, we demonstrate specific interaction between the ectodomain of MIK2 and the elusive elicitor-activity found in our preparations, which further supports the hypothesis that MIK2 functions as a receptor for an exogenous *Fusarium* elicitor. Through further analysis of the retained fractions, the specific interaction between the ectodomain of MIK2 and the elicitor-active molecule could potentially also be utilised to get closer to identifying the true nature of the elusive ligand.

REFERENCES

Coleman AD; Maroschek J; Raasch L; Taaken FLW; Ranf S; Hückelhoven R (2021). The Arabidopsis leucine-rich repeat receptor-like kinase MIK2 is a crucial component of early immune responses to a fungal-derived elicitor. *New Phytologist* 229, 3453-3466.

Shu LJ; Schäffer M; Eschrig S; Ranf S (2021). Low cost, medium throughput depletion-binding assay for screening of S-domain-receptor ligand interactions using in planta protein expression. *bioRxiv* doi.org/10.1101/2021.06.16.448648.

V1-4: Application of an inducible promoter for analysis of virulence factors during *Magnaporthe oryzae* infection *in planta*

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The fungus *Magnaporthe oryzae* is an economically important plant pathogen infecting several crop plants including rice, barley and wheat. Its significance and easy to handle cultivation under laboratory conditions has stimulated extensive research leading to development of a whole set of genetic tools to dissect plant-pathogen interactions. A key objective along this research is the characterization of virulence factors. To this end, we aim to extend the molecular tool box by establishing a method for controlled regulation of gene expression *in planta* during the infection process of *M. oryzae*.

Therefore, the copper-responsive promoter *pFCR1* from *Fusarium graminearum* was introduced in *M. oryzae,* allowing induction of strong gene expression in a copper-free environment that can be turned off by supply of copper. The functionality of this assay was confirmed using fungal mutants containing the *pFCR1* promoter fused to a gene encoding for fluorescent protein. Thus, fluorescence was only detectable in the absence of copper and addition of µmolar copper concentrations effectively inhibited expression of the fluorophore.

For further verification of the system, the *M. oryzae* mutant Δ*Moalb1* was complemented with an intact copy of *ALB1* under the control of *pFCR1*. The deletion mutant is disturbed in its melanin production, which results in growth of whitish mycelium and apathogenicity. In the absence of copper, the active promoter in the complemented mutants led to an overexpression of *ALB1* and restored both above mentioned, as evidenced macroscopically by a greyish mycelium and verified by qPCR. Conversely, when cultivated in copper-containing media, the complemented mutants displayed the phenotype of the deletion mutant. Importantly, studies showed that also pathogenicity of this mutant can be regulated *ad planta* by varying copper concentrations during the stage of spore production. Depending on copper availability, infection of mutants was either regular or blocked, demonstrating the potential for practical applications of this novel tool for *M. oryzae* research.

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V1-5: Non-coding RNAs as Mediators of Beneficial Plant Symbioses

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Non-coding (nc) RNAs are known regulators of development and stress responses in many organisms, including plants and microbes. Interestingly, ncRNAs are, in general, not translated to proteins, but instead expressed to regulate messenger (m) RNA levels and activity. Within the well-known plant-pathogen cross-kingdom communication, small (s) ncRNAs are transferred in two directions; from plants to microbes to regulate virulence/pathogenesis gene expression, and from microbes to plants to suppress and modulate immunity genes (Weiberg *et al*., 2013; Zhang *et al*., 2016). We study ncRNA-based cross-kingdom communication in plant-symbiont interactions and sequenced sRNAs and mRNAs of the broad host range endophyte *Serendipita indica* during colonization of a model grass *Brachypodium distachyon* (Šečić *et al.*, 2021). Combining ncRNA-seq for sRNA, long non-coding (lnc) RNA and circular (circ) RNA detection with comparative ncRNAomics analyses of a diversity of plant-*S. indica* symbioses, our aim is to elucidate ncRNA functions in the expression dynamics of genes involved in establishment of the symbioses and in mediating beneficial effects on plant growth and stress responses.

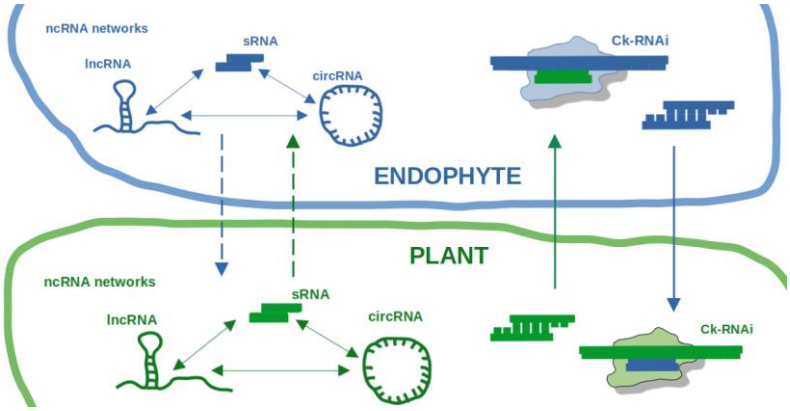

Figure 1: Prospective ncRNAomics in plant symbioses

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V1-6: Ancient variation in avirulence effectors underlies the rapid resistance breakdown of two introgressed rye resistance genes in wheat

Müller MC[1,2*], Kunz L[2*], Schudel S[2], Lawson AW[3], Sotiropoulos AG[2], Graf J[2], Bourras S[2], Keller B[2]

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Crop wild and domesticated relatives are valuable sources of new resistance gene specificities against fungal plant pathogens. The durability of such resistance gene introgressions is highly variable, a phenomenon that remains poorly understood mainly because the corresponding avirulence effectors are unknown. Until their breakdown, the resistance genes *Pm8* and *Pm17*, located on independent rye to wheat translocations, conferred resistance against the wheat powdery mildew disease caused by *Blumeria graminis* f.sp. *tritici*. We used genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping in mildew to identify the corresponding *AvrPm8* and *AvrPm17* effectors both encoding small, secreted proteins that are highly expressed during the early stages of infection. Diversity analysis in powdery mildew collections from major wheat growing areas as well as related powdery mildew lineages revealed that several gain-of-virulence variants of *AvrPm17* and one variant of *AvrPm8* are likely ancient and predate the introgressions of *Pm17* and *Pm8* from rye to wheat. We concluded that standing genetic variation can underlie rapid resistance breakdown of introgressed resistance genes. Our studies demonstrate the relevance of pathogen-based genetic approaches to understand resistance gene durability. We, therefore, argue that the effort to identify durable resistance genes cannot be dissociated from studies of pathogen avirulence genes.

VORTRAGS-SESSION 2

V2-1: Maize phytocytokines and microbial-patterns trigger antagonistic features in co-incidence with wounding and fungal pathogens

Maurice König[1]*, Daniel Moser[1,2]*, Julian Leusner[1], Jasper Depotter[1], Gunther Doehlemann[1,2+] and Johana Misas Villamil[1,2+]
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Phytocytokines are described as signalling peptides activating immune responses and alarming bystander cells. The perception and activation pathways of phytocytokines are thought to be shared with exogenous danger signals such as microbe-associated molecular patterns (MAMPs) and endogenous, passively released, damage-associated molecular patterns (DAMPs). However, it is still largely undiscovered which downstream responses are triggered by different danger molecules into distinct immune responses.
We have identified three biologically active maize orthologues of phytocytokines previously described in other plants. Maize responses to these phytocytokines have common features with MAMPs, i.e. the induction of immune related genes and the activation of papain-like cysteine proteases (PLCPs). However, while MAMPs are promoting cell death in the presence of wounding, this characteristic is not observed for the phytocytokines. Comparative infection assays with biotrophic *Ustilago maydis* and necrotrophic *Botrytis cinerea* revealed that phytocytokines affect the development of disease symptoms, possibly due to the activation of phytohormonal pathways.
Overall, our results show that phytocytokines and MAMPs trigger unique and antagonistic features of plant immunity. Therefore, we propose a model in which phytocytokines activate immune responses partially similar to MAMPs, but in contrast to microbial signals, they act as danger and survival molecules to the surrounding cells. Future studies will focus on the components determining the divergence of signalling outputs upon phytocytokine activation.

V2-2: Adressing redundant roles of phytotoxic proteins of *B. cinerea* for necrotrophic infection by multi-k.o. mutagenesis

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Botrytis cinerea is a necrotrophic fungus infecting a wide range of host plants. During invasion, it rapidly kills host cells and colonizes dead tissue. Factors that contribute to this lifestyle include secretion of CWDE, phytotoxic proteins (CDIPs) and metabolites, tissue acidification and induction of host defence. However, the relative contributions of these factors, and the sequence of events that lead to successful infection and plant cell death are poorly understood. An efficient CRISPR/Cas9 protocol was established, which allowed to generate a series of up to 22-fold multiple mutants, lacking all currently known CDIPs. The mutants showed normal growth and in vitro differentiation, but decreased virulence with increasing numbers of deleted genes. The effects of the deletions were dependent on the origin of the infected tissue, indicating host-specific roles of some CIDPs. The 21x mutant showed strongly impaired lesion formation on leaves, and was virtually nonpathogenic on fruits. Our data document one of the first systematic approaches to address functional redundancy of virulence factors in a pathogenic fungus.
Necrotophic fungi have been shown to induce the plant hypersensitive response (HR) as an infection strategy, and it has been suggested that the CDIPs contribute to HR induction by overactivating plant immune receptors and pattern-triggered immunity (PTI). By using a combined cytological and molecular approach, including the use of *B. cinerea* CDIP mutants and plants defective in PTI and effector-triggered immunity pathways, we attempt to unravel how plant cell death is induced during the infection process, and how does it contribute to fungal infection and plant defence.

V2-3: Licence to kill: *Botrytis* Hip1 alters plant immunity to defeat its hosts

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Necrotrophic pathogens secrete an array of cell wall-degrading enzymes, proteases and toxins. However, their precise role during infection is unknown. Here, we report on the identification and characterization of the previously unknown *Botrytis cinerea* toxic protein hypersensitive response-inducing protein 1 (Hip1), which induces plant cell death.

Localization and the induction of typical plant defense responses by Hip1 indicate recognition as a pathogen-associated molecular pattern (PAMP) at the plant plasma membrane. In contrast to other secreted toxic Botrytis proteins, the activity of Hip1 does not depend on the presence of the receptor-associated kinases BRI1-associated kinase 1 (Bak1) and suppressor of BIR1-1 (Sobir1). Our results demonstrate that recognition of Hip1, even in the absence of obvious enzymatic or pore-forming activity, induces strong plant defense reactions eventually leading to plant cell death. Recent data show that impaired intracellular immune signalling abolishes Hip1 toxicity. In line with this, ROS assays reveal that ROS generation and necrotic activity are tightly linked.

Future work will aim at the identification of the Hip1 receptor/target using a combination of biochemical and genetic approaches (Genome-Wide Association Studies; GWAS).

V2-4: Hyphal fusions enable efficient nutrient distribution in *Colletotrichum graminicola* conidiation and symptom development on maize

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Zea mays is the most grown cereal for human and animal nutrition, which makes detailed knowledge about maize-pathogen interactions essential for food security. The hemibiotrophic fungus *Colletotrichum graminicola* is a maize pathogen infecting several plant tissues like leaves, stems, and roots. Recently, we demonstrated a positive correlation between germling fusion and the formation of penetrating hyphopodia on maize leaves outgoing from *C. graminicola* oval conidia (Nordzieke et al 2019).

The formation of hyphal and germling fusion enables a coordinated development, efficient nutrient distribution and proper interaction of filamentous fungi with plant hosts. To investigate the probable interconnectivity of cell fusion and hyphopodia formation, we generated a deletion mutant in *Cgso*, in which homologs are essential for cellular fusion in other fungal species. However, hyphopodia development of *C. graminicola* was not affected, indicating that both processes were not directly connected. Instead, we were able to link the cellular fusion defect in ΔCgso to a decreased symptom development on leaves caused by a reduced formation of asexual fruiting bodies. The monitoring of a fluorescent-labelled autophagy marker, eGFP-CgAtg8, revealed a high autophagy activity in the hyphae surrounding the acervuli. From these results, we concluded that the efficient nutrient transport of degraded cellular material by hyphal fusions enables proper acervuli maturation and, therefore, symptom development on maize leaves infected with *C. graminicola*.

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V2-5: Molecular analysis of a fungal hybrid of *U. maydis* and *S. reilianum*

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The smut fungi *Sporisorium reilianum* and *Ustilago maydis* are closely related, have similar genomes and infect the same host, *Zea mays*. Like the majority of the smut fungi, *S. reilianum* infects its host systemically and in a later infection stage replaces the inflorescences by teliospores. In contrast, *U. maydis* infects aerial parts of the plant and forms large tumors on maize leaves and the inflorescences. However, the underlying mechanism of the different disease development of *U. maydis* and *S. reilianum* remains unknown.

We exchanged the mating type genes between *U. maydis* and *S. reilianum* and used interspecific hybridization to investigate how the two species contribute with their different effector gene expression to virulence. The recombinant hybrid is able to colonize maize and reveals a *S. reilianum*-like phenotype. Next, we used RNAseq to get insights in the evolution of the different disease development on gene expression level and genome compatibility. Thereby, we identified 218 differently expressed 1:1 effector orthologues in the hybrid which we grouped into distinct gene expression patterns: (a) consistent expression – orthologues which showed the same expression in the wild type and the hybrid and (b) reversed expression – orthologues which are oppositely expressed in the hybrid compared to the wild type. Remarkably, the majority of the reverse expressed effector genes in the hybrid remain in effector gene clusters associated to virulence. Therefore, we hypothesize that *U. maydis* effector genes which showed a downregulation in the hybrid could play a role in tumor formation. To test this idea, infection assays with knock-out mutants of the respective effector genes are performed. Moreover, RNAseq analysis revealed 78 differently expressed transcription factors between *U. maydis* and *S. reilianum*. To assess their putative role in virulence, knock-out mutants were generated and disease symptoms are analyzed. Ultimately, this study aims to show whether the differential effector gene expression between the two species is the molecular basis of the different disease development.

V2-6: The histone lysine methyltransferase Ash1 of *Sporisorium reilianum* negatively regulates effector gene expression in axenic culture

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Smut fungi are biotrophic plant pathogens that colonize their host plants by induction of effector genes. To investigate a putative involvement of epigenetic regulation in effector gene expression, we tested the importance of the putative histone 3 lysine 36 (H3K36) methyltransferase ASH1 for host plant infection of the maize smut fungi *Sporisorium reilianum* f. sp. *zeae* and *Ustilago maydis.* Deletion of the *ASH1* gene in *S. reilianum* led to a change in cell morphology from lemon-shaped to filamentous. The filamentous cells were septated, monokaryotic and haploid, were not able to infect plants as single strains, and displayed reduced virulence when co-inoculated with their compatible mating partner. In contrast, deletion of *ASH1* in *U. maydis* did not affect cell morphology or virulence. Using histone lysine methylation-specific antibodies, we could show absence of H3K36 di- and trimethylation in *S. reilianum* but not *U. maydis* strains lacking *ASH1*. RNA-Seq comparison revealed that lack of *ASH1* induced the expression of 60% of all predicted effector genes in axenically grown *S. reilianum*. In *U. maydis*, lack of *ASH1* led to expression of at least four effector genes under axenic growth conditions. The lack of *ASH1* in *S. reilianum* could be fully functionally complemented by expression of *ASH1* of *S. reilianum* and *Sporisorium scitamineum*, but not by *ASH1* of *U. maydis* or *Ustilago hordei*. Thus, effector expression is controlled by epigenetic mechanisms in smut fungi, with functional variation in the role of the H3K36 methyltransferase ASH1 in different smut fungal species.

V2-7: The monothiol glutaredoxin Grx4 is involved in iron sensing, secondary metabolism, fungal cell surface functions and virulence in *Ustilago maydis*

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The basidiomycete *Ustilago maydis*, the causal agent of corn smut disease, is a model for biotrophic plant-fungal interactions and is also used to investigate fungal cellular processes. Monothiol glutaredoxins (GRX) are key regulators of fungal metabolism. GRXs are involved in iron and redox homeostasis via interactions with iron-responsive transcription factors such as Aft1 or Cir1 from yeast or *Cryptococcus neoformans*. Further, iron homeostasis or redox status is maintained via interactions of Grx4 with iron-sulfur clusters and glutathione.

Here, we report the characterization of Grx4 in *Ustilago maydis.* We initially found that Grx4 is essential in *U. maydis*. Thus, we constructed a glucose-repressible and arabinose-inducible allele by promoter swapping of the wt with the P_{crg} promoter in the FB1 and FB2 strain backgrounds. The EC_{50} value for P_{crg}*:grx4* promoter activation was 0.012% arabinose to allow a 50% growth rate in the presence of glucose. On arabinose no differences were seen for wt or Grx4-depleted strains. RNAseq of glucose repressed and arabinose induced P_{crg}*:grx4* strains was then conducted. Identified Grx4-controlled functions such as iron metabolism (*sid1*; *sid2*), organic acid uptake (*jen2*; j*en20*), cell surface changes (*rep1*), melanin (*mtf1*), mating (*bE*; *bW*) and virulence (*ztf1*; *fox1*) were further investigated. Repression of P_{crg}*:grx4* by glucose could partially be rescued by supplementation with iron, heme or glutathione. Furthermore, the biotrophy-mimicking media condition of glucose plus malate also increased the growth of Grx4-depleted strains. Siderophore secretion was not detectable and cell surface hydrophobicity during mating was reduced in Grx4-depleted strains. In contrast, melanin formation after extended cultivation could be observed in Grx4-depleted cultures. Finally, the regulated strains showed reduced mating and were unable to cause disease on maize seedlings thus indicating a requirement for Grx4 during biotrophic development. Taken together, we identified conserved and new functions of Grx4 in *U. maydis* compared to other fungi.

V2-8: Potential function of anionic phospholipids in HvRACB-supported susceptibility to *Blumeria hordei*

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Plant pathogens use different mechanisms to infect their hosts. Besides suppression of plant immunity via pathogen-derived effector proteins, pathogens exploit host proteins, also called susceptibility factors, for their advantage. One of such susceptibility factors is the barley small ROP GTPase RACB that supports epidermal cell penetration by Blumeria hordei (Bh), the causal agent of the barley powdery mildew disease. Protein-membrane anchoring is crucial for RACB's function in supporting Bh (Schultheiss et al. 2003; Weiß et al. 2022) suggesting that RACB must act at the plasma membrane to fulfil its function in susceptibility. The exact molecular mechanisms underlying RACB-mediated susceptibility in the barley-Bh-pathosystem, however, are not yet known. We now found that activated RACB interacts with plant and fungal proteins that bind or metabolize anionic phospholipids that reside in the plasma membrane of barley epidermal cells. Using a RACB mutant (RACB-5Q), we show that the positive charge of RACB's C-termainal polybasic region is necessary for its membrane attachment. However, the mutant's capabilities to interact with the ROP scaffold protein RIC171, which is also involved in enhancing susceptibility towards Bh (Schultheiss et al. 2008), remained intact. Furthermore, RACB-5Q lost the ability to bind to different anionic phospholipids in vitro. Moreover, some signaling lipids, RACB, RIC171 and other RACB-binding proteins accumulate at the haustorial entry site of penetrated cells. Data reveal a possible link between anionic phospholipids and RACB in host support of fungal ingrowth into barley epidermal cells.

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V2-9: A conserved enzyme of smut fungi facilitates cell-to-cell extension in the plant bundle sheath

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Abstract:
Smut fungi comprise one of the largest groups of fungal plant pathogens causing disease in all cereal crops. They directly penetrate host tissues and establish a biotrophic interaction. To do so, smut fungi secrete a wide range of effector proteins, which suppress plant immunity and modulate cellular functions as well as development of the host, thereby determining the pathogen's lifestyle and virulence potential.
The conserved effector Erc1 (**e**nzyme **r**equired for **c**ell-to-cell extension) contributes to virulence of the corn smut *Ustilago maydis* in maize leaves but not on the tassel. Erc1 binds to host cell wall components and displays 1,3-β-glucanase activity, which is required to attenuate β-glucan-induced defense responses. Here we show that Erc1 has a cell type-specific virulence function, being necessary for fungal cell-to-cell extension in the plant bundle sheath and this function is fully conserved in the Erc1 orthologue of the barley pathogen *Ustilago hordei*.

VORTRAGS-SESSION 3

V3-1: Efficacy of new fungal and bacterial BCAs against Fusarium Head Blight in wheat for NOcsPS cropping systems

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Plant protection is an essential aspect to manage diseases and pests that can result in reduced yields, poor quality, and even crop loss. However, the use of chemical-synthetic plant protection products (csPPPs) is being increasingly criticized due to concerns over their impact on human health and the environment. The new NOcsPS cropping system as expansion to conventional and organic farming systems can offer a promising and environmentally-friendly alternative. NOcsPS allows the use of mineral fertilizers but renounces csPPPs. In order to implement such a NOcsPS farming system, biological control agents (BCAs) have to be established as substitutes for csPPPs.

Several fungal and bacterial BCAs (*Trichoderma* sp. T10, *T. harzianum* T16, *T. asperellum* T23, *Clonostachys rosea* CRP1104, *Bacillus subtilis* HG77 and *Pseudomonas fluorescens* G308) were tested for their efficacy to control Fusarium Head Blight (FHB) in wheat in field trials over the last three years. Application of the BCAs was done twice during anthesis (BBCH 59-69). FHB infection was either artificially (2020) or naturally (2021 and 2022) established.

In 2020, all tested BCAs showed a promising efficacy against FHB in spring wheat with a significant reduction of FHB incidence compared to the pathogen inoculated control. Particularly T16 and CRP1104 showed the best effects, as FHB incidence did not significantly differ from that of the control, that was not inoculated with *F. graminearum*. For T10, HG77 and G308, the similar FHB incidence levels (higher than T23) were recorded. Furthermore, all four fungal BCAs resulted in a significant reduction of kernel infection with *Fusarium* compared to the *Fusarium* inoculated control. Also, in 2022, a significant reduction of FHB incidence could be observed for all BCAs in winter wheat compared to the untreated control.

V3-3: Microbial Biological Control Agents (MBCAs): Consumers' friends or foes?

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V3-4: Urease inhibitors for soil microorganisms reduce severe disease symptoms of fungal pathogens on crop leaves

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The development of new anti-ureolytic compounds is of great interest not only because of the widespread consequences of ureolysis for human health, but also due to the newly discovered urease inhibitor's role in crop protection. Purine degradation and generation of ammonium by urease is required for full virulence of biotrophic and hemibiotrophic fungal plant pathogens. Accordingly, chemicals displaying urease inhibitor activity may be used as a novel class of fungicides. Several urease inhibitors belonging to different chemical classes are known, and some novel compounds have been developed as urea fertilizer-protecting agents. We tested whether novel urease inhibitors of distinct chemical classes can be applied to prevent or delay plant infections caused by pathogens differing in lifestyles and host plants. The quinone-based compounds benzoqionine and hydroquinone not only protected maize from infection with the hemibiotroph *C. graminicola*, but also inhibited the infection process of biotrophs such as the wheat powdery mildew fungus *B. graminis* f. sp. *tritici* and the bean rust fungus *Uromyces viciae-fabae*. Interestingly, these inhibitors even reduced the severity of symptoms of the tested necrotrophic fungi, i.e. the grey mold pathogen *B. cinerea* and the Southern Leaf Spot fungus *C. heterostrophus*, to some extent. Microscopy revealed that the inhibitors tested interfered with appressorial penetration competence and confirmed the appropriateness of urease inhibitory as novel fungicidal agents.

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V3-5: Adaptation of the sensitivity of *Alternaria solani* towards different fungicides

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Early blight, caused by *Alternaria solani*, has the potential to reduce quality and yield in potato production globally. Quinone-outside inhibitors (QoIs), succinate dehydrogenase inhibitors (SDHIs) and demethylation inhibitors (DMIs) represent three major fungicide groups that are used to control early blight on potato. Applications of fungicides have the effect of selection pressure on fungal populations, forcing them to evolve resistance mechanisms to overcome the fungicide treatment. Alteration of the target site of the fungicide, overexpression of the target site and exclusion of the fungicide from the cell are three major mechanisms that may consult in fungicide resistance in pathogens.

Alternaria solani has acquired resistance to QoIs by a target site mutation and to SDHIs by various other target site mutations. Therefore, sensitivity monitoring for QoIs and SDHIs is mandatory for efficient disease and resistance management. In addition, DMIs play an important role for the control of this disease and are therefore exposed to increased selection pressure. Sensitivity to DMIs has also been monitored worldwide using microtiter tests and show a rather stable situation so far.

V3-6: Combining Data-driven deep learning model and Environmental Epidemiology knowledge for prediction Cercospora Leaf Spot in Sugar Beet using multispectral sensors and weather stations

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Early and accurate detection of plant diseases is essential to assess the need for fungicide application and avoid yield losses. In sugar beet, one of the most important is the Cercospora leaf spot disease (CLS), caused by *Cercospora beticola* Sacc. The interaction between the plant and the pathogen influences the optical properties of above-ground sugar beet organs, which can be measured by combine spectral sensors with machine learning approaches. However, weather conditions, environmental factors, and their interaction greatly influence the epidemiological development of the disease and need to be considered in a holistic monitoring approach. For CLS, infection rate is impacted by two main parameters: temperature and relative humidity. However, today, those factors have never been considered in an image-based approach for early and accurate disease detection of CLS. From 2020 to 2022, field trials were conducted near Göttingen, Germany, to investigate the pathogen's spread and its interaction with the environment. Immediately after sowing, spot-inoculation was conducted with *Cercospora beticola*. Georeferenced IoT microclimate sensors were installed to quantify temperature and humidity around the field. In addition, multispectral images (blue, green, red, red edge, and near-infrared and long-wave infrared) were taken weekly from sowing to harvest with a camera mounted on an unmanned aerial vehicle (UAV). At the same time, a visual assessment of the respective disease severity (DS) was carried out to serve as reference data. With the data generated, models were trained to analyze the hybrid combination of optical and environmental information. The training of the models with the environmental data improved the classification obtained by the optical sensors. This work aims to analyze the relevant parameters for the prediction of CLS epidemics and assess the potential of deep learning models to combine data from different sources for future CLS detection work.

V3-7: Leaf analysis for quantitative resistance and disease control using multispectral UAV images: case of Cercospora leaf spot in sugar beet

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Cercospora leaf spot (CLS) is one of the most significant yield-limiting diseases in sugar beet production. Therefore, implementing breeding programs for host resistance, and timely and side-specific control are the principles for managing disease spread. Traditionally, monitoring and disease quantification are performed by trained staff. More recently, optical sensors and sophisticated data analysis approaches are in the focus of research studies to substitute time consuming disease quantification routines. In the last years, unmanned aerial vehicle (UAV) and multispectral imagery have been implemented to phenotype parameters automatically for disease quantification such as disease severity (DS). However, the principal parameter for disease control, disease incidence (DI), requires delimitating scoring units to leaf regions by image analysis. Therefore, we address the challenge of leaf segmentation from multispectral UAV-based images of CLS infested fields by training instance segmentation models (Mask R-CNN). After modeling, the best leaf segmentation approach was identified, and it was applied for time-series disease quantification of a CLS resistance experimental field. Results of instance segmentation modeling highlighted the influence of environmental conditions on model performance. Performance metrics showed that multispectral UAV images recorded under sunny conditions lead to a drop of up to 7% of average precision (AP) compared to images under cloudy, diffuse illumination conditions. UAV-based time-series analysis of disease parameters firstly displays DI for disease control measurements and secondly demonstrates the advantages of DS for contrasting varieties for resistance. With this work, we highlight key components for automatic leaf segmentation of diseased plants using UAV imagery and offer a tool for delivering leaf-based parameters relevant to optimize crop production through automated disease quantification imaging tools.

VORTRAGS-SESSION 4

V4-1: Coordination of cell surface immunity and N limitation by CEP-mediated signalling

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Endogenous signalling peptides regulate a wide range of plant physiological responses. They are involved in the control of plant growth, development and adaptations to biotic and abiotic stress. Recent research indicates that some endogenous peptides have regulatory functions both in growth and immunity. In analogy to metazoan systems, these peptides are referred to as phytocytokines.

We identified specific members of the C-TERMINALLY ENCODED PEPTIDE (CEP) family as novel immune-modulatory peptides in *Arabidopsis thaliana*. CEP application triggers hallmark pattern-triggered immunity (PTI) signaling outputs. Peptide overexpression and loss of function studies confirmed a critical role of CEPs in immunity against infection by *Pseudomonas syringae* pv. *tomato*. CEPs are perceived by leucine-rich repeat receptor-like kinases CEP RECEPTOR 1 (CEPR1) and CEPR2. We provide evidence that CEPR1/CEPR2 also sense immune-modulatory CEPs and are crucial components of antibacterial disease resistance. Yet, CEPR1/CEPR2 are only required for a subset of CEP-induced immune outputs and we will present evidence for a novel CEP receptor.

CEPs have a known role in the control of root growth and systemic N-demand signalling. We now show that CEPs are additionally involved in controlling a previously undescribed cross-talk between N limitation and PTI. We propose that CEPs are central regulators integrating growth and N limitation with biotic stress responses to safeguard plant health.

V4-2: VPg carrying members of the virus yellows diseases complex interact with different translation initiation factors of sugar beet

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The virus yellows disease complex (VY) is an increasing problem for European sugar beet growers and is caused by a complex of different aphid transmitted viruses, namely beet yellows virus (BYV, Closterovirus), beet mosaic virus (BtMV, Genus Potyvirus), beet mild yellowing virus (BMYV) and beet chlorosis virus (BChV) (both Polerovirus). All poleroviruses and potyviruses carry a viral protein genome-linked (VPg) at their 5´genomic end, functioning as mRNA cap-analog for translation initiation. Numerous studies have shown potyvirus VPg interaction with different eukaryotic translation initiation factors (eIFs) of their respective host plants. A recessive resistance to infection is achieved if the VPg-eIF interaction is abolished by knock-out or specific mutations. Initial studies in *Arabidopsis thaliana* support that this concept might be used for polerovirus control as well (Reinbold et al. 2013). However, closely related virus species can depend on different eIFs and even further the same virus can depend on different eIFs in different hosts, making it impossible to directly transfer previous findings to sugar beet (Jiang & Laliberté, 2011). Using a yeast two hybrid (YTH) assay and bimolecular fluorescence complementation (BiFC) different sugar beet eIF4E/4G isoforms were evaluated for interaction with BMYV- and BChV-VPg, as well as with the BtMV-VPg. The VPgs of all species analysed showed an interaction with sugar beet Bv-eIF(iso)4E in BiFC and YTH. Interestingly, the BMYV VPg displayed an additional interaction with the functional redundant eIF4E isoform in BiFC and YTH. After potential interaction partners were identified, T0 knockout sugar beets were generated by genome editing for the different eIFs and subjected to a resistance bioassay. Resistance was obtained against BChV lowering the viral titer significantly, if eIF(iso)4E was knocked out. Interaction for BMYV-VPg with Bv-eIF(iso)4E and Bv-eIF4E give first hints that potentially a double knockout of respective eIFs would be needed to obtain resistance against BMYV, however knocking out resprective eIFs simultaneously is assumed to be lethal for the sugar beet. Therefore, identification of eIF mutants that allow mRNA translation but do not bind to viral VPg is required for future resistance generation.

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V4-3: Assessment of the defense stimulus induced by UV-C light in *Agrostis stolonifera*

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Creeping bentgrass (*Agrostis stolonifera*) is a cool-season turfgrass that is widely used in golf courses. It is severely affected by fungal pathogens. The aim of this work is to develop a device for the application of low energy UV-C radiation to induce protection against pathogens through the stimulation of plant defenses in turfgrass. This device shall be used as a substitute for chemical fungicides in turf care.

Treatment of *A. stolonifera* with UV-C light induces the expression of the defense-related genes *PR3*, and *NPR1* (non-expresser of PR genes) starting 15 min after treatment and reaching maximum levels at 30 min after treatment. Upregulation was observed for up to 48 h in UV-C-treated grass. No apparent change was observed in untreated controls. A dose of 90 mJ/cm^2 for 3 sec was administered twice a week for four weeks. The expression of the targeted defense-related genes was analyzed by Real-time PCR (RT-qPCR). *TEF1*, translation elongation factor 1 alpha, was used as reference gene, and the relative expression of defense-related genes was evaluated using the GenEx software and the ΔΔCt method.

The transient up-regulation in expression of defense-related genes in UV-C-treated grass indicates induced resistance and activation of the corresponding genes. Thus, UV-C radiation induces the transcription of genes involved in the defense against pathogens. It was also seen that UV-C irradiation twice a week for 3–4 weeks was more effective than a single treatment. These *in vitro* findings correspond to reduced disease incidence after UV-C treatments of turfgrass on a golf course.

V4-4: An avirulence effector from barley powdery mildew fungus can suppress *Mildew locus A* resistance.

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The barley powdery mildew fungus *Blumeria hordei* (*Bh*) relies on a huge repertoire of effectors for successful infection and the development of disease symptoms on the barley host. In resistant lines, the *Bh AVR$_a$* effectors are recognised by cognate barley nucleotide-binding leucine-rich repeat receptors encoded at the *Mildew locus A* (MLA NLRs). For example, AVR$_{A13}$-1 is recognized by MLA13. The effector-receptor interaction activates host cell death, and this terminates powdery mildew disease development. We demonstrate that a virulent form of AVR$_{A13}$, called AVR$_{A13}$-V2, escapes MLA13 recognition by substituting a serine for a leucine residue at the C-terminus. Counterintuitively, this substitution in AVR$_{A13}$-V2 results in an enhanced MLA13 association and prevented the detection of AVR$_{A13}$-1 by MLA13. Therefore, AVR$_{A13}$-V2 is a dominant-negative form of AVR$_{A13}$. Our interaction analysis involving effector and receptor mutant variants constitute an important step to define intermediate receptor conformations during NLR activation and implies that the dominant-negative function of AVR$_{A13}$-V2 has contributed to the breakdown of *Mla13* resistance.

V4-5: Analysis of codon usage and allele frequencies reveal the double-edged nature of cross-kingdom RNAi

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BACKGROUND

In recent years, a new class of small 21- to 24-nt-(s)RNAs has been discovered from microbial pathogens that are transferred to interfere with their hosts' gene expression during infection, in a process called cross-kingdom RNA interference (ckRNAi). According to this model, microbial sRNAs should exert selection pressure on plants so that host gene sequences that reduce complementarity to sRNAs are preferred. To test this ckRNAi model changes in codon usage and allele frequencies of target sequences were analysed in the model systems *Arabidopsis thaliana* (*At*) – *Hyaloperonospora arabidopsidis* (*Ha*) and *Hordeum vulgare* (*Hv*) – *Fusarium graminearum* (*Fg*). Interestingly, taking the degree of complementarity to pathogen sRNAs as scalable measure, both sets of microbial sRNAs were found to have a tendency towards codons with a high instead of an expected lower degree of complementarity. To distinguish between complementarity caused by pathogen adaptation vs. plant adaptation, the host population structure was analysed, revealing the directional selection of complementary codons.

These findings suggest an evolutionary pressure to facilitate silencing by exogenous microbial sRNAs, which is not consistent with the anticipated biological role of pathogen sRNAs as exclusively effectors in cross-kingdom RNAi. To resolve this conflict, we propose an extended model in which microbial sRNAs are perceived by plants via RNA interference and, as a result of co-evolution, primarily help to fine-tune plant gene expression.

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V4-6: Non-coding RNAs as smart bio-fungicides in future crop protection

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RNA interference (RNAi) is a biological process in which non-coding RNA (ncRNA) molecules, such as microRNAs (miRNAs) and small interfering RNAs (siRNAs), sequence-specifically silence gene expression at the transcriptional or post-transcriptional level. ncRNAs act either by directing inhibitory chromatin modifications or by decreasing the stability or translation potential of the targeted mRNA. The primary trigger for ncRNA formation and gene silencing is double-stranded RNA (dsRNA) generated from an endogenous genomic locus or a foreign source, such as a virus or artificial exogenous dsRNA. Given the high potential of RNAi strategies in disease control combined with the absence of ecotoxic effects, their application in agriculture, horticulture, and forestry will likely be extensive in the future. Rapid progress in elucidating RNAi mechanisms has led to first commercial products on all global markets, except for Europe.

Among pathogens affecting crop yield, fungi form the largest and most diverse group. Due to their huge diversity in colonizing host plants, it is highly challenging to find a single strategy to tackle all of them. Here, we present our major findings on the antifungal activity of dsRNA, their uptake and RNAi-based gene silencing activity in two different pathogenic fungi, *Verticillium longisporum* and *Magnaporthe oryzae*. We present a workflow developed in our lab that, starting with *in vitro* experiments to its final application *in planta*, is effective in identifying dsRNAs reducing fungal diseases. We discuss advantages and disadvantages of the RNAi technology to control pest and pathogens, through different methods of application of *in vitro* synthesized dsRNA.

V4-7: AGRIFUTURE: a tool for rapid plant pathogen detection using mobile nanopore sequencing

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Pathogens can cause serious losses in agriculture, leading to economic damage and posing a threat to global food security. Global trade of agricultural products and a changing climate further enable the spread of pathogens into previously unfavourable regions. Therefore, detecting pathogens rapidly before they are spreading to new regions is critical for crop protection. Nanopore sequencing technology has enabled the rapid, on-site taxonomic identification of samples from anything and anywhere. However, sequencing errors, inadequate databases, as well as the need for bioinformatic expertise and powerful computing resources, have hampered the widespread adoption of the technology for pathogen identification in the agricultural sector. Here, we present a lightweight and accurate real-time taxonomic profiling pipeline for portable sequencing and pathogen detection from plant samples. Compared to other metagenomic profilers, the pipeline had a higher classification precision, achieved through the use of a curated, non-redundant genomic database of common agricultural pathogens and extensive quality filtering of alignments. The pipeline is available as a stand-alone tool and as a web application with live basecalling at http://agrifuture.senckenberg.de**.**

POSTER-SESSION 1

P1-2: The effector *Mo*Nudix is required for full virulence of *Magnaporthe oryzae* on barley

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The rice blast disease is caused by the hemibiotrophic fungus *Magnaporthe oryzae,* which in addition to rice, also infects staple food crops like barley, millet and wheat, making it a significant threat to global food security. To successfully infect plants, the pathogen secretes proteins, referred to as effectors, which manipulate the host immune response. To gain deeper insight in this process, the function of *MoNUDIX,* a gene encoding a nudix-hydrolase, was studied. Nudix-hydrolases are known to cleave a wide variety of nucleoside di- and triphosphates linked to organic substrates like e.g. ADP-Ribose, NADH or m^7Gppp caps of ribonucleic acids and are known to play an important role in plant defense and fungal infection processes. Transcripts of the gene *MoNUDIX* were found to peak around 48 hours post inoculation, suggesting a role for the switch from the biotrophic to the necrotrophic lifestyle. Confocal Laser Scanning microscopy of an *in locus* mRFP-tagged mutant revealed the accumulation of the fusion protein in the biotrophic interfacial complex (BIC), a specialized cellular region of infection hyphae enriched with secreted fungal proteins. Deletion of both copies of the gene in the *M. oryzae* isolate Guy11 resulted in a significant loss of virulence on barley. This results were verified using CRISPR/Cas9 technology for a marker-free *in locus* complementation and reversion to the wild-type infection phenotype of respective mutants. Interestingly, this reduction in virulence was not observed on rice, potentially pointing to a role as determinant for host-specificity. Further analysis of published genomes revealed the absence of orthologous genes in wheat-isolates. Conversely, the gene was present in all other pathotypes of *M. oryzae*. Next, we aim to identify the natural substrate for this protein using recombinant expression to gain insight into the molecular function of this novel effector.

P1-3: Protein pull down and proteomic analysis of the SAD1 smut effector protein and its interacting proteins from *Arabidopsis thaliana* and *Zea mays* for improved cob productivity in maize

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Abstract:

The number of inflorescences of plants is of economic importance. In the horticultural industry, a higher number of inflorescences may be desired, while a lower number of larger inflorescences is a trait highly preferred for industrially-harvested crop plants. The number of inflorescences in plants is strongly influenced by apical dominance. The effector protein SAD1 (Suppressor of Apical Dominance1) from the smut fungus *Sporisorium reilianum* f. sp. *zeae* is involved in early inflorescence branching by inducing loss of apical dominance in infected maize (*Zea mays*) and in transgenic *Arabidopsis thaliana*. To understand the molecular involvement of the fungal effector protein SAD1 with plant inflorescence development, we used antibodies raised against putative epitopes of SAD1 in pull down experiments. In a first step, SAD1 protein and its *in-planta* interaction partners were copurified by co-immuno precipitation from transgenic *A. thaliana* that stably expresses the fungal protein in all cells. The pull down proteins were then subjected to Liquid Chromatography – Mass Spectrometry (LC-MS) analysis. We will present information on found interacting plant proteins as well as on putative post-translational modifications of SAD1. Understanding the molecular role of SAD1 on plant inflorescence development will facilitate strategically influencing the number of plant inflorescences in the desired direction, for example for improved cob productivity in maize.

P1-4: Establishing RNA spray-based solutions for sustainable control of the biotrophic fungal pathogens *Phakopsora pachyrhizi* and *Blumeria graminis* f.sp. *hordei*

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The use of chemical synthetic fungicides is the first choice for controlling fungal pathogens incrops because of their high efficacy. However, the biodiversity crisis and the development of resistance are the main reasons why alternatives are urgently needed. RNAi-based crop protection offers a highly selective and promising alternative, using double-stranded RNA to interfere post-transcriptionally with gene expression. Targeted gene silencing can be achieved by genetically modified plants (host-induced gene silencing), which produce dsRNA through transgene expression, or by exogenously sprayed dsRNA (spray-induced gene silencing). Our previous research has demonstrated the efficacy of HIGS and SIGS in controlling the necrotrophic fungal pathogen *Fusarium graminearum*. In particular, the barley-*F. graminearum* pathosystem showed a high sensitivity to dsRNA sprays. We predict that this is due to the necrotrophic lifestyle of *F. graminearum*, which enables the uptake of long, unprocessed dsRNA from the plant apoplast and its processing by the fungal RNAi machinery. In contrast, biotrophic phytopathogens rely on RNA uptake from the symplast together with nutrient acquisition. This raises the question of whether biotrophs can be controlled by RNA spraying as effectively as necrotrophs. To this end, two biotrophic fungi, *Phakopsora pachyrhizi* and *Blumeria graminis* f.sp. *hordei*, will be tested for their sensitivity to dsRNA spraying and whether biointelligent RNA formulations that increase cellular uptake can enhance RNAi-mediated disease resistance in barley and soybean. If RNA sprays can be established against biotrophic fungi, it will provide a revolutionary solution to reducing the use of conventional chemical pesticides.

P1-5: Enrichment and characterization of an Asian Soybean Rust PAMP and identification of two loci involved in the perception in *Arabidopsis thaliana*

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P1-6: Flow cytometric purification of *Magnaporthe oryzae* infected barley cells for transcriptome analysis

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Plants rely on defense mechanisms of individual cells after attack by a pathogen. Even in a compatible interaction, some cells resist penetration or colonization, while others are successfully infected by the pathogen. In order to understand genetic and metabolic processes, which are different in infected and resistant cells, a selective analysis of gene expression profiles of individual cells or populations, rather than of whole-leaf samples, is mandatory.

Using the *Magnaporthe oryzae* – barley pathosystem, we aimed to develop a strategy by which barley cell populations colonized by the fungus can be enriched and separated from those that are not yet attacked using Fluorescence Activated Cell Sorting (FACS). For this purpose, *M. oryzae* mutants were generated constitutively expressing a fluorophore. Primary leaves were inoculated with these mutants and used for generation of protoplasts. We established an advanced protocol to separate mesophyll from epidermal protoplasts and sorted the latter based on the fluorescence signal of the fungal hyphae by using FACS. Next, RNA-sequencing of the different cell populations is aimed to be performed. Finally, the gene expression profiles of the infected and not-infected cells will be compared in order to identify both, barley genes that are either exclusively or differentially expressed in infected vs. non-infected cells, and fungal genes, that are expressed during plant colonization. Potential candidate genes will subsequently be characterized using different molecular tools.

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P1-7: Volatile-mediated signaling in barley induces metabolic reprogramming and resistance against the biotrophic fungus *Blumeria hordei*

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Plants have evolved diverse secondary metabolites to counteract biotic stress. Volatile organic compounds (VOCs) are released upon herbivore attack or pathogen infection. Recent studies suggest that VOCs can act as signalling molecules in plant defence and induce resistance in distant organs and neighbouring plants. However, knowledge is lacking on the function of VOCs in biotrophic fungal infection on cereal plants. We analysed VOCs emitted by 13 day-old barley plants (*Hordeum vulgare* L.) after mechanical wounding using passive absorbers and TD-GC/MS. We investigated the effect of pure VOC and complex VOC mixtures released from wounded plants on the barley–powdery mildew interaction by pre-exposure in a dynamic headspace connected to a powdery mildew susceptibility assay. Untargeted metabolomics and lipidomics were applied to inv estigate metabolic changes in sender and receiver barley plants. Green leaf volatiles (GLVs) dominated the volatile profile of wounded barley plants, with (Z)-3-hexenyl acetate (Z3HAC) as the most abundant compound. Barley volatiles emitted after mechanical wounding enhanced resistance in receiver plants towards fungal infection. We found volatile-mediated modifications of the plant–pathogen interaction in a concentration-dependent manner. Pre-exposure with physiologically relevant concentrations of Z3HAC resulted in induced resistance, suggesting that this GLV is a key player in barley anti-pathogen defence.
The complex VOC mixture released from wounded barley and Z3HAC induced e.g. accumulation of chlorophyll, linolenic acid and linolenate-conjugated lipids, as well as defence-related secondary metabolites, such as hordatines in receiving plants. Barley VOCs hence induce a complex physiological response and disease resistance in receiver plants.

P1-8: A UAV based Monitoring System for the early Detection of Plant Diseases in Field Vegetable Cultures

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Our subproject six of DiWenkLa cooperative project evaluates the possibilities of establishing a drone-based monitoring system for the early detection of plant diseases and pests in field vegetable cultures in Southwest Germany. A Micasense Dual Camera Sytem mounted to an UAV was used for data collection in the field.

In order to be able to identify infested spots in an area within the multispectral image data, it was necessary to improve the accuracy of the optical monitoring workflow with which the ground truth data were collected. A highly accurate GPS-device in combination with the qField GIS-application was used to achieve the required accuracy. As a nice side-effect data collection could be completely digitalized in the process. Results from the first vegetation period were diverse but promising. For the second vegetation period our spectrum of crops analyzed was extended for strawberries and wheat. The sensor setup was also changed and extended by including a thermal and a high resolution RGB-sensor. Results to date indicate that for a resilient disease detection system image data (from all different sensors) need to be combined with additional data like weather- or forecast model data.

P1-9: Diversity of *Peronospora* causing downy mildew disease on *Myosotis* and *Veronica*

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Myosotis and *Veronica* contain both weedy and ornamental plants of commercial value. Both genera are widespread throughout the northern hemisphere. For both genera, more than a dozen species are known to be attacked by downy mildew, including economically relevant ornamentals, such as *Myosotis sylvatica*. However, the biodiversity of *Peronospora* on *Myosotis* and *Veronica* is poorly investigated, with only one species validly published from *Myosotis* and eight species validly published from *Veronica*. For gaining first detailed insights into the diversity of *Peronospora* on these host genera, 101 *Peronospora* specimens from *Myosotis* and *Veronica* were phylogenetically investigated based on two nuclear loci (ITS, LSU) and four mitochondrial loci (*cox2*, *cox1*, *nad1*, *rps10*) and also microscopically scrutinized for morphological differences. As a result, six new *Peronospora* species infecting *Myosotis* and seven new *Peronospora* species parasitic on *Veronica* were revealed. Interestingly, most *Peronospora* species seem to affect only a single host species, but two had larger host ranges. However, the specimens we studied are just the tip of the iceberg, because *Myosotis* has about 60 species and *Veronica* has around 500 species, several of which are known to affected by downy mildew. This work, thus, highlights the need for further studies on the species diversity in the genus *Peronospora* infecting Plantaginaceae and Boraginaceae to enable risk assessment and targeted phytosanitary measures to prevent economic loss.

P1-11: Detection of the soybean pathogen *Diaporthe* spp. in soil

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Fungi from the genus *Diaporthe* cause important soybean diseases. Recently, we established that the four species *D. longicolla*, *D. caulivora*, *D. eres*, and *D. novem* are relevant in Germany and we established a quadruplex real-time PCR to detect them.

Diaporthe species are generally considered to be seed borne. However, it is also possible that the fungi endure on crop residues and in soil and infect plants in the next year. To elucidate this and for disease prevention, we now pursue detection of these species in soil using our quadruplex real-time PCR.

Soil samples were infested with serial dilutions of spore suspensions of *D. longicolla* and *D. eres*. 100 mg of the inoculated soil was used for DNA extraction and the DNAs were tested in the real-time PCR. *D. longicolla* and *D. eres* could be detected successfully in the soil samples.

Based on our dilution series we have established a rough limit of detection for the two species in soil. Also, a time course experiment is ongoing with samplings after one hour, one day, one week, and afterwards once a month. This should provide information about the survival of *Diaporthe* spores in the soil. In addition, we have started sampling soybean fields and are testing soil samples adjacent to infected soybean plants.

If our experiments indicate that soil infestation can be problematic, we will complement the real-time PCR detection with experiments testing soybean plants grown in infested soil.

P1-12: Sea Buckthorn Dieback in Northern Germany: Results of Fungal Community Sequencing

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Sea buckthorn (*Hippophae rhamnoides* L.) is a dioecious shrub grown on an area of 596 ha in commercial plantations in North-East Germany (Statistisches Bundesamt 2022). Further, plants along the coastline of the Baltic Sea serve for erosin protection. Plants in both habitats are increasingly affected by dieback symptoms, resulting in serious losses of up to 100 %. External symptoms comprise wilting of single branches with dried-out leaves and fruits, shoots shrinkage, black-reddish discoloration and lesions of the bark, and discolorations are evident in cross sections of the shoot. Up to now, the exact cause of the final plant death remains unknown. A joint-project, HippRham, started in 2020 to reveal the cause of sea buckthorn dieback and to develop possible control strategies. As result from an extensive isolation approach, *Hymenopleella* and *Diaporthe* were the most frequently isolated fungal genera from symptomatic shoot samples, both not identified in asymptomatic samples.

In addition to the classical 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. DNA extracts generated for 151 shoot, 86 root and 70 soil samples derived from different locations were used for ITS1 metabarcoding. Here we report on results of these mycobiome analyses.

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1-13: Inhibition of *Alternaria solani* and *Phytophthora infestans* through volatile organic compounds emitted by *Trichoderma* spp.

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P2-1: A powdery mildew fungus hijacks endogenous FER-RALF signaling to establish successful host colonization

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With over 400 different species and nearly 10 000 possible host plants, powdery mildew is one of the most widespread fungal diseases. During the powdery mildew infection cycle, a constant interaction occurs between the fungus and the host plant which determines the infection success. Plant genes that support pathogen infection are termed susceptibility genes. In Arabidopsis, the receptor kinase FERONIA (FER) was found to be a powdery mildew susceptibility gene, as fer mutants are more resistant to infection. FER has previously been shown to perceive RAPID ALKALINIZATION FACTOR (RALF) peptide ligands to control various aspects of plant growth, development and immunity. With a combination of peptide treatment, loss-of-function and overexpression studies, we now show that RALF sensing by FER is required for successful powdery mildew infection on Arabidopsis. We hypothesize that FER-RALF regulated alterations of the apoplastic pH support powdery mildew growth.

Currently, we are trying to unravel FER-RALF signaling pathways facilitating powdery mildew susceptibility and study the cell biology of FER during powdery mildew infection.

P2-2: Comparative metabolomics of *Solanum lycopersicum* to elucidate anti-fungal defence mechanisms

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Plants employ a multi-layered innate immune system to defect and fight off invading pathogens. In addition to well-studied responses such as a Reactive Oxygen Species (ROS) burst and strong up-regulation of ethylene, salicylic acid, jasmonic acid, and other phytohormones, the Secondary Metabolites (SM) are expected to be involved in quantitative resistance of different plants to certain pathogens. Tomato plants inoculated with *Alternaria solani* show clear symptoms of infection after 5 days of inoculation. But plants inoculated with *Alternaria alternata* do not show any symptom after several days of inoculation. We hypothesized that induction of resistance related metabolites in *Solanum lycopersicum* that maintains the resistance, yet successful suppression of such SM in a susceptible interaction. We compare the metabolomic profile of *S. lycopersicum* at two different time points after treatments with *A. alternata* (CS046), *A. solani* and Chitin. The latter is a general elicitor of anti-fungal plant defence responses. We subsequently identify SMs that can be induced in the early defence response in tomato plants. Our study revealed different chemotypes depending on the treatment and time point. A total of 68 candidate metabolites associated with resistance in tomato plants were identified. Increased levels of an alkaloid, trigonelline, were detected after treatments with chitin and *A. alternata* and inhibition of the fungus growth *in vitro* when supplied trigonelline at physiological concentrations was observed. Our findings indicate the presence of induced chemical defense barriers against *A. alternata*.

P2-3: Cascade use of lupin plants: protein for food and feed and source for high-value chemicals

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The project P³roLucas (Optimization of plant performance and products for lupin cascade use) is a joint project between three research groups from Universities and the Forschungszentrum Jülich, based in the state of North Rhine-Westphalia, Germany, with the aim of promoting and improving lupin cultivation.

Lupin is a valuable alternative protein source suitable for human food and animal feed and presents an attractive alternative to (GMO-)soybean from international markets. Due to intensive breeding efforts, nowadays more varieties with improved agronomic traits, including tolerance against anthracnose disease are available, but yield potential is varying between species.

Species of the genus Lupinus are known for their alkaloid content, which is reduced in the so-called “sweet” varieties, but high in “bitter” varieties, which in turn are more resistant to diseases. Alkaloid content must be monitored because of its negative impact on human and animal health. There is a process of debittering established in which alkaloids are removed from bitter varieties and up to now they are treated as waste. However, from a chemist point of view alkaloids are of interest, because they contain highly valuable and rare (pre)chiral compounds such as lupanin (2-oxosparteine) and sparteine.

In this project explore the use of biostimulants and plant strengtheners in lupin production, for modulating alkaloid production, improving yield and increasing pathogen- and abiotic stress-tolerance. Plant responses will be investigated on the biochemical and transcriptomic level, using consolidated and, in frame of this project, newly generated genomic resources (*L. mutabilis* genome) as a basis. In an exemplary cascade use approach, we want to demonstrate additional benefits of lupin production, by using the hitherto wasted alkaloids as a source for high value chemicals. Additionally, producer’s acceptance of the entire concept will be assessed.

P2-4: Analysing the role of the *Botrytis cinerea* phytotoxic secretome for necrotrophic pathogenesis

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Botrytis cinerea is a necrotrophic plant pathogen with a wide host range. During invasion, the fungus quickly kills host cells and colonizes dead tissue. Factors shown to contribute to infection are plant cell wall degrading enzymes, cell death inducing proteins (CDIPs), phytotoxic metabolites and induction of plant defence responses such as the hypersensitive response (HR).

Based on a highly efficient CRISPR/Cas9 protocol, we have generated a series of up to 22-fold multiple mutants that are lacking all currently known CDIPs. The mutants showed normal growth and differentiation, but strongly reduced infection on leaves, and almost no infection of fruit tissue, indicating a highly redundant role of CDIPs for necrotrophic infection.

During infection, *B. cinerea* secretes a highly phytotoxic secretome into the host tissue, which can be recovered from inoculation droplets after lesions have formed. The secretome of the 22x mutant had lost >80% of its toxic activity compared to the WT secretome. An MS-based secretome analysis confirmed the loss of the deleted CDIPs. By applying a combined bioinformatic and biochemical approach, we are searching for the remaining CDIPs that are involved in host cell killing. This will allow us to generate *B. cinerea* mutants completely devoid of CDIPs, and to elucidate the role of CDIPs for necrotrophic infection.

P2-5: The Pec effector complex of *Ustilago maydis* interferes with carbohydrate metabolism in maize

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Ustilago maydis is a biotrophic smut fungi that infects all aerial parts of *Zea mays and secretes a cocktail of* effectors in a spatiotemporally regulated manner to form cell-type specific leaf tumors in maize. The infection caused by cell-type specific effectors produces highly nutrient-rich tumors and interferes with the host's metabolism to redirect carbon flux towards the increased accumulation of starch and soluble sugars as well as to alter the transition from sink to source leaves.

Using a cell-type-specific transcriptome profiling of *U. maydis* during tumor formation, a set of specifically and strongly upregulated effector genes during hypertrophy which causes enlargement of mesophyll cells upon infection was revealed [(1)] Among ten mesophyll specific effectors, two effectors (Pec1 and Pec2- **P**rimary metabolite **E**ffector **C**omplex) are required for full virulence. Furthermore, a deletion mutant of Pec3, a paralogue of Pec2 identified in subsequent mass spectrometry (MS) analysis, also showed a significant reduction in tumor formation.

To investigate how Pec effectors act on a molecular level, immunoprecipitation followed by mass-spectrometry analysis from *U. maydis* infected maize extracts was performed. To our surprise, we found that the Pec1-3 effectors interact with each other inside the plant tissue. The possible effector complex was tested in Co-immunoprecipitation (Co-IP) and Split-luciferase complementation experiments. This showed that Pec effectors are interacting with each other. Moreover, Pec1 interacts with maize SNF1 (Sucrose Non-Fermenting 1) related protein kinase, which plays an important role in regulating cellular energy metabolism.

To elucidate if Pec effectors act cooperatively in the plant, we conducted a large-scale MS analysis, using Pec effector deletion mutant strains of effectors complemented on wild-type background. This analysis revealed that Pec1 is the central effector of those putative effector complex. To better understand Pec effectors' role on primary metabolism, starch and soluble sugars were quantified in infected maize leaves. In plants infected with *U. maydis* Pec single-, double-, and triple knock-out mutants, we found reduced and mis-localized starch accumulation in comparison to wild-type infected plants, indicating that Pec effectors act cooperatively to reprogram metabolic changes in maize.

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P2-6: Wheat diversity set analyses reveal genotype-specific priming capacities induced by beneficial endophytes

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Beneficial microorganisms can trigger a response in plants named priming, which is a durable condition allowing plants a fast and effective immune response against pathogens and pests. In addition, it 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 is a prerequisite for developing new breeding traits that could significantly reduce the use of pesticides and fertilisers.

In this study, we analyzed the priming response of wheat *(Triticum aestivum)* as a function of genetic diversity. Beneficial fungal and bacterial endophytes such as *Serendipita indica* and *Rhizobium radiobacter F4* served as priming inducers. The priming capacity (biomass, yield, induced resistance) was investigated in a genetically highly diverse set of selected winter wheat genotypes. Genotypes with higher capacity for endophyte-mediated priming responses were identified on 4-week-old plants where we found high phenotypic variability for the priming trait.To demonstrate the agronomic applicability of microbe/iome-induced priming, experiments under field and semi-field conditions have been conducted. In addition, a GWAS (genome-wide association study) analysis of the generated data will be used to elucidate the genetic basis of priming.

P2-8: Deciphering host plant penetration of the pathogenic fungus *Botrytis*

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The necrotrophic fungus *Botrytis cinerea* secretes large amounts of phytotoxic proteins and cell wall-degrading enzymes to kill host plant cells. However, the actual mode of host cell entry is not well understood and the precise force applied by (pseudo) appressoria is largely unknown. Here, we use a combination of high resolution microscopy, surface-deformation imaging and mathematical modelling to understand the biomechanics of host entry by *Botrytis*. We observed a polarized mechanical geometry which is surprisingly similar to the slicing “naifu” mode of entry previously observed in Phytophthora (Bronkhorst et al., 2021). From measuring the depth of indentation in combination with the radius of invasive organ we could calculate invasive pressures (based on the Herzian contact mechanics model). We found pressures of around 4 bars in the wild type but also in higher-order mutants lacking important secreted phytotoxic proteins. Using mutants impaired in virulence will allow us to address to which extend geometry of penetration and the applied invasive pressure contributes to the pathogenicity of *Botrytis*.

P2-9: Proof of concept for spectral phenotyping of Syndrome Basses Richesses in sugar beet using hyperspectral sensors and machine learning

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The “syndrome basses richesses” (SBR) of sugar beet is one of the most recent sugar beet diseases described in literature and heavily increasing in economical relevance[1]. SBR is predominantly caused by the γ-3-proteobacterium “Candidatus Arsenophonus Phytopathogenicus“, a phloem-limited prokaryote which can cause cell necrosis and lignification in sugar beet phloem cell walls[1–3]. Though, little is known about the host-pathogen-interaction. Moreover, the phenological changes of neither the tap root nor the canopy have been quantified yet. Therefore, inoculation trials under controlled conditions have been used to improve knowledge on symptom development and phenological stages of SBR diseased sugar beet plants. Greenhouse cultivated sugar beet plants of two different varieties have been inoculated with the proteobacterium loaded insect vector, *Pentastiridius leporinus*. In addition to visual monitoring and destructive investigations, hyperspectral measurements were used for digital phenotyping since visible and non-visible alteration of leaves cause changes in the light reflectance properties which can be quantified via non-invasive spectral sensors. Furthermore, biophysical and biochemical attributes of plant vegetation can be derived from certain reflectance signatures of leaves[4]. To establish spectral signatures of SBR-diseased sugar beet plants, time-series measurements over three months with an imaging (500-1000 nm) and a non-imaging (300-2500 nm) hyperspectral sensor were performed. Spectral signatures of SBR-diseased plants were extracted and compared to a healthy control. The data is currently analyzed with different machine learning algorithms to identify relevant spectral features and establish a model for the prediction of developmental stages of the disease.

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P2-10: Mechanisms of basidiomycete yeast function in complex leaf microbial communities

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Plants are colonized by a multitude of microorganisms, which interact not only with its host, but also show complex interaction networks within the microbial community. In 2016, a scale-free high resolution network analysis of the leaf microbiome of *A. thaliana* revealed that many of the interactions are negative (1).
The oomycete pathogen *Albugo laibachii* identified as a key microbe in shaping the microbial community. It is inhibited by the basidiomycete yeast ***M****oesziomyces* ***b****ullatus ex Albugo on* ***A****rabidobsis* (short: *MbA*), which significantly reduces the virulence of *A. laibachii* (2). To understand the role of *MbA* in the *A. thaliana* phyllosphere and to find potentially involved genes in the inhibition of *A. laibachii*, RNA sequencing was performed. This approach identified *MbA* candidate genes encoding putative secreted proteins out of which 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 unravel the mechanisms by which GH25 functions and to explore its evolutionary conversation. Adressing the evolutionary conversation, the GH25 of the smut fungus *Usilago maydis* is being explored, which shows 77% sequence similarity to *MbA* GH25. Preliminary data indicates a limited inhibition of *Albugo laibachii* by *U. maydis* GH25. To this point, the function of GH25 could not be linked to an activation of the plant immune system. Hence, the modulation of the microbial community and inhibition of its members might be a mode of action of GH25. Indeed, one member of the bacterial community that is closely related to *A. laibachii* is inhibited by GH25. Ongoing experiments aim to elucidate if such multipartite antagonistic interactions are causal for the inhibition of *A. laibachii* by GH25.

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P2-11: Impact of cultivar resistance on *Cercospora beticola* epidemiology on sugar beet

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Cercospora leaf spot (CLS), caused by the fungal pathogen *Cercospora beticola*, is the most destructive foliar disease on sugar beet (Holtschulte, 2000). With the emergence of fungicide-resistant populations (Rangel et al., 2020), the importance of developing and cultivating resistant cultivars is increasing. Understanding the interactions between the cultivar resistance and *C.beticola* is essential in CLS management. A field experiment was designed from 2022 to 2023 to investigate these interactions from an epidemiological aspect. More specifically, this field trial aimed to describe the relationship between the cultivar resistance and the spore flight of *C.beticola*. The trials were carried out in two geographical locations as a completely randomized block design (RCBD) with four cultivars containing different resistant properties in triplicate. In the trial in 2022, spore flight during the vegetation period was examined for all four cultivars by a pre-developed method using spore traps and TaqMan real-time PCR assay (Imbusch, 2020). Disease development on each cultivar was also monitored during the same period. We observed a delayed disease incidence and decreased disease severity in highly resistant cultivars compared to the susceptible and moderately resistant varieties. The result of the TaqMan real-time PCR analysis for spore flight shows a similar tendency of reduced spore quantity sampled from the highly resistant cultivars. A correlation between spore quantity and disease development was further confirmed. The results from this trial indicate that the highly resistant cultivars were able to produce fewer secondary aerial spores, which further decreased the disease development. This trial will be performed again in 2023. We expect our result will give a better insight into the interaction between cultivar resistance and CLS epidemiology.

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P2-12: Searching powdery mildew-resistant oaks

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Oaks (pedunculate and sessile oaks) make up ca. 10 percent of the forest tree population in Germany. Oak powdery mildew (OPM) caused by *Erysiphe alphitoides* is the most significant oak disease in Europe and one of the most prominent 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 (Bartholomé et al., 2020). The overall goal of the project is to examine silvicultural, physiological, cytological and molecular biological evidences of mildew-resistant oaks.

By using primers based on the internal transcribed spacer region of 18S rRNA gene, we have found that there is very limited diversity within OPM population in Kaiserslautern region. We have established protocols for staining of OPM with Calcofluor white, Coomassie brilliant blue and wheat germ agglutinin Oregon Green™ Conjugate.

We have optimized an OPM spore suspension inoculation protocol for the study of infection on susceptible and resistance oak using detached leaves. The formation of elongating secondary hyphae (ESH) is a reliable indicator of successful haustorium formation and plant colonization by powdery mildew. Our preliminary results show that inoculation of OPM on leaves of resistant red oak at 48 hours post infection did not show any ESH while on susceptible sessile oak leaves, many spores had formed ESH.

In the related project Survivor-Oak a comparative transcriptome analysis of healthy and PM-infected oak leaves was conducted, and 75 genes were found to be up-regulated upon PM infection. We started RT-PCR analysis by selecting 23 genes that were significantly up-regulated and that encoded proteins commonly involved in plant defence responses.

Reference: Bartholomé, J. et al. (2020) The genetics of exapted resistance to two exotic pathogens in pedunculate oak. New Phytol. 226, 1088–1103.

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P2-13: Maize phytocytokines modulate pro-survival host responses and pathogen resistance

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Phytocytokines are signaling peptides that alarm plant cells of danger. However, the downstream responses triggered by phytocytokines and their effect in plant survival is still largely unknown. Here, we have identified three biologically active maize orthologues of previously described phytocytokines. The maize phytocytokines show common features with microbe associated molecular patterns (MAMPs), including the induction of immune related genes and activation of papain-like cysteine proteases. In contrast to MAMPs, phytocytokines do not promote cell death in the presence of wounding. In infection assays with two fungal pathogens we found that phytocytokines affect the development of disease symptoms, likely due to the activation of phytohormonal pathways. Collectively, our results show that phytocytokines and MAMPs trigger unique and antagonistic features of immunity. We propose a model in which phytocytokines activate immune responses partially similar to MAMPs but in contrast to microbial signals, they act as danger and survival molecules to the surrounding cells. Future studies will focus on the components determining the divergence of signaling outputs upon phytocytokine activation.