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6. März 2020

Tagung der DPG-Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“ am 19./20. März 2020 an der TU München

Sehr geehrte Damen und Herren, liebe Kolleginnen und Kollegen,

wir laden Sie hiermit herzlich zur gemeinsamen Tagung der Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“ 2020 ein. Beigefügt erhalten Sie das Programm für beide Arbeitskreise und die Abstracts der Beiträge, sofern vorhanden.

Tagungsort ist das Wissenschaftszentrum Weihenstephan der TU München, Gebäude 4277 Forstwissenschaften, Hörsaal 21/22, Erdgeschoß, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising. Ein Dokument mit Lageplan haben wir Ihnen zugeschickt.

Beginn der Vorträge ist am Donnerstag, 19.03.2019 um 13:00 Uhr

Am Donnerstagnachmittag tagen beide Arbeitskreise gemeinsam, am Freitagvormittag zunächst getrennt. Für jede/n Referentin/en sind 12 Minuten Redezeit und 3 Minuten Diskussionszeit vorgesehen. Wir bitten Sie, die Zeiten einzuhalten und die Vorträge gut einzuführen, damit auch Nicht-Spezialisten davon profitieren können.

Die Poster drucken Sie bitte im DIN A0-Format.

Im Anschluss an die Vorträge findet am Donnerstag-Abend ein geselliges Beisammensein statt im Bräustüberl Weihenstephan, Weihenstephaner Berg 10; 85354 Freising. Nähere Informationen dazu werden bei der Tagung mitgeteilt.

Wir wünschen Ihnen eine gute Anreise und freuen uns auf ein interessantes Treffen mit spannenden Präsentationen und regen Diskussionen.

Mit freundlichen Grüßen

Anne-Katrin Mahlein und Matthias Hahn

Deutsche Phytomedizinische Gesellschaft e.V.

PROGRAMM

Donnerstag, 19.3.20 (Beide Arbeitskreise: Hörsaal 21/22)

13:00 Uhr	**BEGRÜSSUNG**
13:10 Uhr	B. Ökmen (Uni Köln): A secreted ribonuclease in biotrophic smut fungi induces cell death in plant
13:25 Uhr	D. Spencer (RWTH Aachen): Engineered Phylloplane Targeting of Antifungal Coumarins for Plant Protection
13:40 Uhr	M. Stegmann (TU München): Identification of novel phytocytokines regulating plant immunity.
13:55 Uhr	S. Bohnert (RWTH Aachen) Rapid adaptation of signaling networks in the fungal pathogen *Magnaporthe oryzae*
14:10 Uhr	A. Djamei (IPK Gatersleben): The Pleiades cluster of fungal effector genes inhibit host defenses
14:25 Uhr	E. Alisaac (Uni Göttingen): Fusarium head blight (FHB) in wheat: effect of infection timing on disease development and mycotoxin accumulation
14:40 Uhr	B. Werner (Uni Giessen): Investigations of sRNAs in the *Fusarium graminearum* - *Hordeum vulgare* - Pathosystem
14:55 Uhr	P. Schäfer (Uni Ulm): Cell identity determines cell type-specific immunity networks in Arabidopsis roots
15:10 Uhr	**KAFFEEPAUSE / POSTER-PRÄSENTATION (Foyer Hörsaal 21/22)**
16:10 Uhr	D. Schenke (Uni Kiel): New insights into the Verticillium – plant interaction: the role of phytohormones and potential susceptibility factors
16:25 Uhr	M. Hoffmeister (BASF, Limburgerhof): New insights into the infection biology of *Peronospora salviae-officinalis* causing downy mildew disease on common sage
16:40 Uhr	Yu Wang (Uni Bonn): Stimulation of *Plasmodiophora brassicae* resting spore germination is induced by the soil microbiome rather than by native root exudates.
16:55 Uhr	A. Passarge (Uni Köln): Characterisation of ryegrass cysteine proteases and their inhibition during *Epichloë festucae* interaction
17:25 Uhr	A. Brugger (Uni Bonn): Hyperspectral imaging in the UV-range of susceptible and resistant barley genotypes inoculated with *Blumeria graminis* f.sp. *hordei*
17:40 Uhr	C. Kirsch (RWTH Aachen): OsJAC1 - Revealing the mode of action of a defense conferring rice protein
17:55 Uhr	T. Schlemmer (Uni Gießen): Towards a RNAi-based control of plant diseases: Research into its mechanistic basis
18:10 Uhr	Hinweise für das gemeinsame Abendessen
ab 19:00 Uhr	**GEMEINSAMES ABENDESSEN im Bräustüberl Weihenstephan, Weihenstephaner Berg 10, Freising**

Deutsche Phytomedizinische Gesellschaft e.V.

Freitag, 20.3.20 (Arbeitskreis Wirt-Parasit-Beziehungen: Hörsaal 21)

8.30 Uhr N. Agrawal (Uni Jena): The *Sporisorium reilianum* effector SAD1 leads to loss of apical dominance by interfering with the function of the plant E3 ubiquitin ligase RGLG1 and RGLG2

8:45 Uhr P. Kahlon (TUM): Diversity in early defence responses in a wild tomato species *Solanum chilense*

9:00 Uhr B. Hosseini (Uni Hohenheim): Multiplex Real-Time PCR for detection of Diaporthe/Phomopsis Complex (DPC) species on soybean

9:15 Uhr A. Raschke (Uni Halle): Iron homeostasis controls cell wall integrity of the maize pathogen *Colletotrichum graminicola*

9:30 Uhr B. Navarro (Uni Göttingen): Charakterisierung der Ht-Resistenzgene in Mais gegen *Exserohilum turcicum*

9:45 Uhr S. Schumacher (Weinbauinstitut Freiburg): Identification and characterization of Nep1-like proteins from the grapevine downy mildew pathogen *Plasmopara viticola*

Freitag, 20.3.20 (Arbeitskreis Mykologie: Hörsaal 22)

8.30 Uhr M. Loehrer (RWTH Aachen): *In vino veritas* - uncovering the secrets of success of the Asian grapevine leaf rust *Phakopsora euvitis* on grapevine

8:45 Uhr V. Küpper (DLR Rheinpfalz) SmartBioS: Smarte Biostimulantien für einen kupferreduzierten Rebschutz

9:00 Uhr A. Barreto (IfZ, Göttingen): Proof of concept for visual rating of Cercospora leaf spot using multispectral UAV image

9:15 Uhr D. Alnajar (Uni Göttingen): Rassenbestimmung lokaler Populationen des Erregers der Rapswurzelhals und -stängelfäule, *Leptosphaeria maculans*

9:30 Uhr A. Pfordt (Uni Göttingen): *Trichoderma harzianum* -Ein neues Pathogen im Mais?

9:45 Uhr F. Hoheneder (TU München) Drought stress affects Ramularia leaf spot disease in an assortment of climate adapted barley genotypes

10-11 Uhr KAFFEEPAUSE / POSTER-PRÄSENTATION (Foyer Hörsaal 21/22)

(Beide Arbeitskreise: Hörsaal 21/22)

11:00 Uhr F. Leißing (RWTH Aachen): Biochemical characterization of MLO2 in Arabidopsis thaliana

11:15 Uhr A. Mantai (RWTH Aachen): Nematode ascaroside ascr#18 primes plants for enhanced defense

11:30 Uhr M. Hahn (TU Kaiserslautern): Advanced CRIPR/Cas technology for fungicide resistance research

11:45 Uhr C. Matera (Uni Bonn): Introducing *Arabidopsis thaliana* as a host for *Cercospora beticola*

12:00 Uhr I. Saur (MPI Köln) Identification of unrelated powdery mildew avirulence effectors and their surveillance by allelic barley Mildew Locus A immune receptor

12:15 Uhr S. Schurack (Uni Köln): Elucidating the molecular basis of quantitative disease resistance in the maize – *Ustilago maydis* interaction

12:30 Uhr L-J. Shu (TU München) Structural and functional analysis of LORE-dependent 3-hydroxy fatty acid immune sensing in Brassicaceae

12:45 Uhr J. Maroschek (TU München): The LRR receptor kinase MIK2 mediates pattern-triggered immunity responses against *Fusarium* species in Arabidopsis.

13.00 Uhr Termin & Ort für Arbeitskreistreffen 2021, Verschiedenes

Poster:

S.F. Beyer (RWTH Aachen): Scopoletin provides plant tolerance to different biotic stresses

A. Coleman (TU München): The Arabidopsis leucine-rich repeat receptor kinase MIK2 is a crucial component of pattern-triggered immunity responses to Fusarium fungi

R. Eichmann (Univ. Ulm): Root growth inhibition induced by the immune elicitor flg22 is mediated by cell cycle arrest

S. Engel (RWTH Aachen): Identification of histone methyltransferases in defence priming

S. Engelhardt (TU München): Barley RIC157 – Building a bridge to facilitate RACB-mediated susceptibility to powdery mildew

S. Eschrig (TU München): Roles of the protein domains of the B-lectin S-Domain receptor kinase LORE in oligomerization and immune signalling

R. Fuchs (Weinbauinstitut Freiburg): VitiMeteo - an internet platform for sustainable viticulture

M. Hoffmeister (BASF Limburgerhof): New insights into the infection biology of *Peronospora salviae-officinalis* causing downy mildew disease on common sage

L. Kutzner (Uni Regensburg): Impact of small secreted maize proteins on *Ustilago maydis* pathogenicity

S. Laupheimer (TU München): (Z)-3-Hexenyl acetate, a volatile organic compound, modulates susceptibility in barley-powdery mildew interaction

T. Leisen (TU Kaiserslautern): CRISPR/Cas9 with ribonucleoprotein complexes allows highly efficient marker-free editing in *Botrytis cinerea*

J. Müller (TU Kaiserslautern): Role of the plant vacuole in plant immunity

L.M. Muñoz (TU München): Comparative profiling of defense-associated secondary metabolites in tomato wild species *Solanum chilense*

A. Passarge (Uni Köln): Characterisation of ryegrass cysteine proteases and their inhibition during *Epichloë festucae* interaction

M. Rieker (Uni Hohenheim): Detection and control of *Fusarium graminearum* and *Sclerotinia sclerotiorum* in the new NOcsPS cropping system

A. Rybecky (Uni Köln): Generation and functional characterization of artificial effector gene clusters in *Ustilago maydis*

P. Schwinges (RWTH Aachen): Rainfast release systems for efficient copper-based crop protection

P. Sengupta (Uni Köln): Mechanisms of antagonism in the leaf microbial community of *Arabidopsis thaliana*

S. Singh (Uni Kiel): *Verticillium longisporum* suppresses plant defensin PDF2.2 to initiate a compatible plant-fungus interaction in *Arabidopsis thaliana*

M. Sonnek (Uni Halle): The H3K4 methyltransferase gene KMT2 is a novel virulence factor of the maize anthracnose pathogen *Colletotrichum graminicola*

D. Spencer (RWTH Aachen): Engineered phylloplane targeting of antifungal coumarins for plant protection

M. Stegmann (TU München): Identification of novel phytocytokines regulating plant immunity

C. Steidele (TU München): Using multi-omic resources to learn more about putative roles of orphan receptor-like proteins in *Arabidopsis thaliana*

B. Thirukonda (Uni Jena): Does histone lysine methylation control host-adapted gene expression in *Sporisorium reilianum?*

S. Thomas (Uni Hohenheim): UAV based hyperspectral imaging combined with modern data analysis for non-invasive disease detection improves efficiency of precision farming

A. Trutzenberg (TU München): Analysis of PRONE GEF14 as a potential activator of ROP GTPases in barley powdery mildew

M. Vega-Marin (Uni Göttingen): Lineage characterization and phylogenetic analysis of *V. longisporum* strains from European and Canadian oilseed rape fields

A. Wegner (RWTH Aachen): Investigating the armory of *Magnaporthe oryzae*: MoPl1 is required for full virulence of *M. oryzae*

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

Tagung der DPG-Arbeitskreise „Mykologie“ und „Wirt-Parasit-Beziehungen“ am 19./20. März 2020 an der TU München

A) Abstracts der Vorträge

B) Abstracts der Poster (S. 25 ff.)

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

Bilal Ökmen, Raphael Wemhöner and Gunther Doehlemann

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In order to successfully colonize their hosts, fungal pathogens establish a complex network of interactions not only with their respective host but also with other organisms present in the environment. These involve production and secretion of effectors to manipulate host defense and metabolism, and secretion of antimicrobial compounds to modulate host microbiota. Ribotoxins are a group of highly specific extracellular ribonucleases that specifically target and cleave the universally conserved sarcin-ricin loop sequence of rRNA, which leads to inhibition of protein biosynthesis and subsequently to cell death.
In this study, we have characterized the RNase T1 which is conserved in all smut fungi. Although smut Ribo1 is more closely related to the non-toxic RNase T1 members, heterologous expression of Ribo1 from *U. hordei*, *U. maydis*, and *Sporisorium reilianum* in *Nicotiana benthamiana* revealed cell death-inducing activity.
To functionally understand the mode of Ribo1-induced cell death, active site mutants of both secreted and non-secreted version of UhRibo1 have been tested. While extracellularly induced cell death is independent from enzymatic activity, cell death induced by intracellular expression requires an active Ribo1 enzyme. Thus, we hypothesize that while the mode of intracellularly UhRibo1-induced cell death is associated with its rRNA cleavage activity, the extracellular UhRibo1 is most likely recognized by a yet unknown plant receptor to induce defense responses. In light of their biotrophic lifestyle, it is tempting to speculate about the biological function of the cell-death inducing Ribo1 proteins in smut fungi.

Engineered Phylloplane Targeting of Antifungal Coumarins for Plant Protection

Spencer D, Schwinges P, Biermann RT, Dreischhoff S, Weber Böhlen J, Beesley A, Beyer SF, Schultheiss H, Conrath U, Langenbach CJG.

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Engineering crops for enhanced accumulation of antimicrobial secondary metabolites is a promising means for sustainable disease management. Here, we show that nonhost

resistance-associated defence mechanisms of sunflower (*Helianthus annuus*) can be exploited for engineered production and secretion of antifungal coumarins to the leaf surface. Heterologous expression of sunflower genes encoding both biosynthetic enzymes and ATP-binding cassette (ABC) transporter proteins resulted in rapid and efficient coumarin export. Transient co-expression of the identified *H. annuus* ABC transporter with different coumarin biosynthetic genes in *Nicotiana benthamiana* revealed its capacity to not only provoke scopoletin export to the phylloplane, but also of the structurally similar coumarin derivative scoparone, hinting to a broad substrate range of the cloned ABC protein. Our results indicate that finetuning the secondary metabolism of crops by exploiting genetic resources found throughout the plant kingdom is a promising strategy for sustainable plant protection.

Identification of novel phytocytokines regulating plant immunity

Martin Stegmann[1], Patricia Zecua-Ramirez, Ralph Hückelhoven[1]

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Tight control of the on and off set of defense responses ensures the proper availability and allocation of cellular resources for plant growth and development. Several classes of endogenous plant signalling peptides play an important role in regulating diverse physiological responses. These peptides can be classified into different groups and are either produced as propeptides requiring subsequent proteolytic activation, with or without additional post-translational modifications, or are produced as mature peptides from small open reading frames. These signalling peptides play an important function in several growth and developmental processes such as root growth, cell expansion, stem cell fate and cell separation events. In addition, in recent years it became evident that several classes of these peptides have regulatory functions in the control of plant immune responses. For example, it has been shown that RALF peptides act as suppressors or activators of PRR-triggered immunity via the FER receptor kinase. Thus, these peptides can be classified as phytocytokines as they share similar functions with animal cytokines.

We mined publically available gene expression data in the model species *Arabidopsis* and found several specific members of plant endogenous peptides with a strong differential transcriptional regulation in response to elicitors and/or pathogen infection, suggesting that they play a role in pathogen susceptibility and/or resistance. Here, we show that overexpression and loss of function of distinct peptides shows altered PRR-triggered immunity and antibacterial resistance. In addition, treatments with chemically synthesized peptides show similar modifications of immunity. We are currently trying to dissect the molecular mechanisms governing the regulation of plant immune responses by these newly identified modulatory phytocytokine peptides.

Rapid adaptation of signaling networks in the fungal pathogen *Magnaporthe oryzae*

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One fundamental question in biology is how the evolution of eukaryotic signaling networks has taken place. “Loss of function” (lof) mutants from components of the high osmolarity glycerol (HOG) signaling pathway in the filamentous fungus *Magnaporthe oryzae* are viable, but impaired in osmoregulation. After long-term cultivation upon high osmolarity, stable individuals with reestablished osmoregulation capacity arise independently from each of the mutants with inactivated HOG pathway. This phenomenon is extremely reproducible and occurs only in osmosensitive mutants related to the HOG pathway – not in other osmosensitive *Magnaporthe* mutants. The major compatible solute produced by these adapted strains to cope with high osmolarity is glycerol, whereas it is arabitol in the wildtype strain. Genome and transcriptome analysis resulted in candidate genes related to glycerol metabolism, perhaps responsible for an epigenetic induced reestablishment of osmoregulation, since these genes do not show structural variations within the coding or promotor sequences. This is the first report of a stable adaptation in eukaryotes by producing different metabolites and opens a door for the scientific community since the HOG pathway is worked on intensively in many eukaryotic model organisms.

REFERENCES

Bohnert, S., Antelo, L., Grünewald, C., Yemelin, A., Andresen, K., & Jacob, S. (2019). Rapid adaptation of signaling networks in the fungal pathogen Magnaporthe oryzae. *BMC genomics*, *20*(1), 763.

The Pleiades cluster of fungal effector genes inhibit host defenses

Djamei A.[A,C], Navarrete F. [A,], Grujic N. [A,], Stirnberg A. [A,], Aleksza D. [A,], Gallei M. [A,], Adi H. [A,], Bindics J. [A,] Trujillo M. [C,]

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Biotrophic plant pathogens secrete effector proteins to manipulate the host physiology. Effectors suppress defenses and induce an environment favorable to disease development. Sequence-based prediction of effector function is difficulted by their rapid evolution rate. In the maize pathogen Ustilago maydis, effector-coding genes frequently organize in clusters. Here we describe the functional characterization of the pleiades, a cluster of ten symplastic effectors. Deletion of the pleiades leads to strongly impaired virulence and accumulation of reactive oxygen species (ROS) in infected tissue. Eight of the Pleiades suppress the production of ROS upon perception of pathogen associated molecular patterns (PAMPs). Although genetically redundant, the Pleiades target different host components. The paralogs Taygeta1 and Merope1 suppress ROS production in either the cytoplasm or nucleus, respectively. Merope1 targets and promotes the autoubiquitination activity of RFI2, a conserved family of E3 ligases that regulates the production of PAMP-triggered ROS burst and influences flowering time in plants.

***Fusarium* head blight (FHB) in wheat: effect of infection timing on disease development and mycotoxin accumulation**

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The effect of infection timing on *Fusarium* head blight (FHB) development and mycotoxins contamination in wheat kernels has been investigated. Two wheat varieties with different degrees of resistance to FHB were inoculated with *Fusarium graminearum* starting from anthesis (0 days after anthesis, daa) until 25 daa. Five 5 µl of inoculum (1×10^5 spore/mL) were injected into the space between the palea and the lemma of the two-terminal florets of the central spikelets. At harvest, the spikes were collected and each spike was parted into three

equal segments: tip, center (which contained the inoculated spikelets), and base. Fungal DNA, deoxynivalenol (DON) and DON-3-glucoside (DON-3-G) were quantified using qPCR and HPLC-MS in the kernels of each part of the spike. Fungal DNA analysis and re-isolation showed that the pathogen grew from the inoculation point downward. The fungus was recovered from the centers and the bases of all spikes. Neither the fungus nor fungal DNA was isolated in the spike tips for all inoculation times and both varieties. The susceptible variety "Sonett" showed higher fungal DNA content compared with the moderately resistant "Triso" in the centers and the bases and all inoculation times. In each variety, fungal DNA content was higher in the centers than in the bases for all inoculation times. Higher amounts of DON and DON-3-G were detected in "Sonett" than "Triso" for the same part of the spike in all inoculation times. In each variety, the centers contained more DON and DON-3-G than the bases in all inoculation times. The ratio of DON-3-G to DON was comparable in both varieties and higher in the bases than in the centers until 15 daa "Sonett" and 10 daa in "Triso". Afterward, the ratio was higher in "Sonett" compared with "Triso". Positive correlation was found between fungal DNA and DON content in the centers as well as the bases of both varieties ranging between r = 0.58-0.85. This study showed that *F. graminearum* grows downwards within infected wheat spikes and that accumulation of DON is largely confined to the colonized tissue. Contrary to expectation, *F. graminearum* was able to infect wheat kernels and cause contamination with mycotoxins even when inoculated 25 days after anthesis.

Investigations of sRNAs in the *Fusarium graminearum* - *Hordeum vulgare* - pathosystem

B. Werner, K.-H. Kogel

Universität Giessen

Cell identity determines cell type-specific immunity networks in Arabidopsis roots

Schäfer P [1], Rich-Griffin C[2], Eichmann R[1], Reitz MU[2], Hermann S[3], Woolley-Allen K[2], Esteban E[4], Pasha A[4], Kogel KH[3], Provart NJ[4], Ott S[5]

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While root diseases are most devastating in global crop production, our understanding of root immunity is still limited relative to our knowledge of immune responses in leaves. Considering that root performance is based on the concerted functions of its different cell types, we

undertook a cell type-specific transcriptome analysis to identify gene networks activated in epidermis, cortex and pericycle cells of Arabidopsis roots challenged with two immunity elicitors, the bacterial flagellin-derived flg22, and the endogenous Pep1 peptide. Our analyses revealed that both elicitors induced distinct immunity gene networks in each cell type. To further substantiate our understanding of regulatory patterns underlying these cell type-specific immunity networks, we developed a tool to analyse paired transcription factor-binding motifs in promoters of cell type-specific genes. Our study points toward a connection of cell identity and cell type-specific immunity networks that might guide cell types in launching immune response according to the functional capabilities of each cell type.

New insights into the *Verticillium* – plant interaction: the role of phytohormones and potential susceptibility factors

Dirk Schenke and Daguang Cai

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Verticillium longisporum infects oilseed rape (*Brassica napus*) and *Arabidopsis thaliana*. To understand these plant-Verticillium interactions, we comparatively analysed transcriptomic changes in response to fungal infection and found that at the early infection stage (6 dpi) the expression of genes related to the ABA-phytohormone pathway clearly distinguished between Arabidopsis and oilseed rape: being significantly supressed in oilseed rape but not in Arabidopsis. We demonstrated that ABA is required for a full susceptibility of Arabidopsis to the fungal infection while the fungus-induced suppression of ABA in oilseed rape might be part of the virulence mechanism, benefiting the fungus to establish a long-lasting compatible interaction with oilseed rape. Furthermore, we developed a strategy to identify potential susceptibility factors and demonstrate that the gene CRT1a encoding Calreticulin is required for the fungus to successfully infect both Arabidopsis thaliana and oilseed rape. Loss-of-function of CRT1a reduced plant susceptibility to *V. longisporum* and might help therefore to protect plants from fungal infection. Interestingly, the loss-of-function of CRT1a enhances the ethylene signaling pathway in both Arabidopsis and oilseed rape, strongly suggesting a sophisticated interplay of the phytohormones to fine tune the plant-Verticillium interaction.

REFERENCES

Behrens FH, Schenke D, Hossain R, Ye W, Schemmel M, Bergmann T, Häder C, Zhao Y, Ladewig L, Zhu W and Cai D (2019): Suppression of abscisic acid biosynthesis at the early infection stage of *Verticillium longisporum* in oilseed rape (*Brassica napus*). Mol Plant Pathol. 20(12), 1645-1661.

Pröbsting M, Schenke D, Hossain R, Häder C, Thurau T, Krapoth L, Schuster A, Zhou Z, Ye W, Rietz S, Leckband G and Cai D (202X): Loss-of-function of CRT1a (Calreticulin) reduces plant susceptibility to *Verticillium longisporum* in both *Arabidopsis thaliana* and oilseed rape (*Brassica napus*). In submission.

New insights into the infection biology of *Peronospora salviae-officinalis* causing downy mildew disease on common sage

M. Hoffmeister[1], W. Maier[2], M. Thines[3,4], Y. Becker[2]

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Downy mildew of common sage, caused by *Peronospora salviae-officinalis*, has become a serious problem in sage production worldwide. *P. salviae-officinalis* is an obligate biotrophic pathogen, which grows in the intercellular spaces of the leaf tissue of sage and forms intracellular haustoria to interface with host cells. Although this pathogen was described already a decade ago, only little is known about its infection biology and epidemiology. The aims of the current study were therefore to unravel the life cycle of this downy mildew and gain deeper insights into the epidemiology of the disease by infection experiments under controlled conditions and confocal laser scanning microscopy (CLSM).

A histological study of various infection events, from the adhesion of conidia on the leave surface until *de novo* sporulation is presented here. As histological studies of oomycetes are challenging due to the lack of chitin in their cell wall, we also present an improved method for staining downy mildews for confocal laser scanning microscopy. For this, we evaluate the potential of the autofluorescence of fixed non-stained samples. A 1:1 mixture of aniline blue and trypan blue was found most suitable and was used for staining of oomycete and plant structures, allowing a distinction between them and the visualization of plant immune responses. The method was also used to examine samples of *Peronospora lamii* on *Lamium purpureum* and *Peronospora belbahrii* on *Ocimum basilicum*. This shows the potential of the presented histological method for studying the infection process of downy mildews in general.

Stimulation of *Plasmodiophora brassicae* resting spore germination is induced by the soil microbiome rather than by native root exudates

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Plasmodiophora brassicae is an obligate biotrophic parasite that causes a devastative soil-borne disease of cruciferous crops known as clubroot. Infected roots become swollen and

distorted that restricts their ability of nutrient and water uptake. Under natural conditions, germination of resting spores occurs spontaneously at low rates but can be stimulated by various biotic and abiotic factors. Native root exudates from both host and non-host plants had no significant stimulatory effects on spore germination *in vitro*. In order to investigate the potential impact of the soil microbiome, aqueous soil extracts from several soil types were tested for their effect in breaking down spore dormancy. The results showed that the germination rate of the resting spores was significantly increased in non-filtered compared to filtered (0.2 µm syringe filter) soil extracts. Several bacterial strains which were isolated from the soil had a stimulatory effect on spore germination. Incubation with selected bacterial isolates induced considerably higher germination rates than the control. These results indicate that the soil microbiome, particularly soil bacteria, probably plays a crucial role in the initiation of *P. brassicae* spore germination. Identification of biochemical signals that stimulate the germination of *P. brassicae* resting spores could provide innovative methods for the integrated sustainable management of this notorious pathogen.

Characterisation of ryegrass cysteine proteases and their inhibition during *Epichloë festucae* interaction

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A growing body of literature highlights the importance of papain-like cysteine proteases (PLCPs) in plant immunity. At the same time more and more pathogen effectors targeting PLCPs or their regulators are being identified. However, so far not much is known about the role of PLCPs and their inhibition in non-pathogenic interactions, such as the Lolium perenne - Epichloë festucae interaction. The cool season grass L. perenne is extensively cultivated for livestock feed and the association with E. festucae enhances its resistance to biotic and abiotic stresses.

We performed a genome wide identification of PLCPs, analysed their evolutionary relationship and classified them into nine PLCP subfamilies. A proteomics approach identified four active PLCPs in the L. perenne leaf apoplast. Remarkably, their activity is strongly inhibited during E. festucae interaction. We found accumulation of a L. perenne derived apoplastic cystatin during Epichloë festucae interaction and characterised its inhibitory capacity on PLCP activity. Moreover, analysis of a E. festucae re-annotated genome allowed the identification of two candidate effectors with a putative PLCP inhibitor function. We propose that the endogenous ryegrass cystatin inhibits PLCP activity during L. perenne – E. festucae interaction and will discuss a possible function of E. festucae derived inhibitors for establishment of symbiotic interaction.

Hyperspectral imaging in the UV-range of susceptible and resistant barley genotypes inoculated with *Blumeria graminis* f.sp. *hordei*

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In recent studies, hyperspectral imaging for plant phenotyping purposes has been extended to the ultraviolet range (UV, 200-380 nm). As a requirement it is assumed that early host-pathogen interactions have an influence on plant substances like secondary metabolites and therefore change the reflection of plants in the UV-range. A hyperspectral imaging setup in the UV-range has been established to highlight the influence of early host-pathogen interactions on plant metabolites.
In this study, time series measurements of healthy barley leaves and barley leaves inoculated with Blumeria graminis f.sp. hordei were conducted. Three genotypes with a high susceptibility as well as resistance due to the formation of cell wall appositions or hypersensitive reactions were used. Changes in the spectral signatures enabled a differentiation between the genotypes and can be related to pathogen and genotype-specific effects on secondary plant metabolites.
In addition, a data set recorded in a high-throughput system was used for deep learning analysis which resulted in a very high predictive accuracy. By looking at the decision process of the deep learning model it became obvious that data points were used which do not relate to the biological problem and expert knowledge has to be used to correct the model.
By extending hyperspectral imaging to the UV-range information on changes in the metabolism of plant substances during pathogenesis and resistance reactions can be recorded.

***Os*JAC1 - Revealing the mode of action of a defense conferring rice protein**

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Members of the *Poaceae* family express proteins with a dirigent and a jacalin-related lectin (JRL) domain. These domains occur in dicotyledonous plants only in separate proteins. Previously, we have shown that overexpression of a JRL from rice (*Os*JAC1), confers broad spectrum disease resistance to bacterial and fungal plant pathogens in rice, barley and wheat. To determine proteins which are additionally required for this resistance response, we tested whether *Os*JAC1 interacts with molecules like e.g. carbohydrates or glycosylated proteins. It was found that galactose containing carbohydrates strengthen *Os*JAC1's stability in a thermal

shift assay and that *Os*JAC1 dimerizes *in vivo* and *in planta*. Additionally, it was observed that *Os*JAC1-GFP overexpressing barley plants are dwarfted as which was reported by Jiang et al. (2007) for *Os*JAC1-overexpressing rice plants. This points either to costs associated with constitutively expression of *Os*JAC1 or to a function of the protein in control of the vegetative growth. In a yeast-two-hybrid screen five putative interaction partners of *Os*JAC1 were identified. Independent assays like split-YFP are currently performed to verify the proposed protein-protein interaction.

Towards a RNAi-based control of plant diseases: Research into its mechanistic basis

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The *Sporisorium reilianum* effector SAD1 leads to loss of apical dominance by interfering with the function of the plant E3 ubiquitin ligase RGLG1 and RGLG2.

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SUPPRESSOR OF APICAL DOMINANCE1 (SAD1) is an effector of *Sporisorium reilianum* that leads to an increase in the number of ears of *S. reilianum*-infected maize plants. By yeast two-hybrid library screening, we identified the E3 ubiquitin ligase ZmRGLG1 as an interaction partner of SAD1. SAD1 interacts with the homologs of ZmRGLG1 (ZmRGLG2, AtRGLG1, and AtRGLG2) in targeted yeast two-hybrid experiments. The in-planta BiFC experiment showed that SAD1 interacts with RGLG1/2 at the nucleus and the cytoplasm. In the RNA-Seq analysis, we found that the presence of SAD1 leads to the upregulation of abiotic stress response genes in maize. The E3 ubiquitin ligases AtRGLG1/2 are known to have a role in abiotic stress response. *rglg1rglg2* Double mutants of *A. thaliana* shows loss of apical dominance. We generated transgenic *A. thaliana* lines expressing mCherry-SAD1ΔSP and lacking or not AtRGLG1 or AtRGLG2. Expression of SAD1 in *rglg1* and *rglg2* single mutants led to an increase in the number of branches displaying suppression of apical dominance. This suggests that SAD1 indeed interferes with the function of both E3 ubiquitin ligases. We performed an in-vitro ubiquitination assay and found that SAD1 can be ubiquitinated by RGLG1/2. We propose that SAD1 interferes with the interaction of RGLG1/2 with their natural targets, such as AtERF53, that is normally targeted for degradation to turn off the prolonged abiotic stress response. This would lead to increased persistence of the stress-responsive transcription factor AtERF53, which will lead to prolonged-expression of abiotic stress response genes and loss of apical dominance.

Diversity in early defence responses in a wild tomato species *Solanum chilense*

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Natural plant populations show a wide range of quantitative resistance phenotypes. The factors contributing to this variation remain poorly understood. We want to understand these factors using *Solanum chilense* as a model system. *S. chilense* is a wild tomato species existing in a wide variety of habitats. These habitats are expected to be home to different pathogens (or strains), which would result in variation in pathogen defence responses. Indeed, phenotypic differences in resistance against *Phytophthora infestans*, *Alternaria solani* and a *Fusarium* sp. can be observed in geographically distinct populations of *S. chilense* (Stam et al., 2017). To link these differences to the responsible factors, we looked into well studied initial immune responses like reactive oxygen species (ROS) burst and accumulation of phytohormones like ethylene and salycilyc acid. It is known in *Arabidopsis thaliana* that ROS burst intensity correlates with resistance against certain pathogens and also defence associated roles of above-mentioned phytohormones have been established previously.

We developed several large-scale screening assays on adult *S. chilense* leaves using laminarin (a cell wall component of oomycetes) as an elicitor. We screened plants from eight different populations, using up to ten individual plants per populations and multiple biological replicates to account for the large phenotypic variation in leaf morphology of adult tomato plants. Surprisingly, whereas we see generic trends when we compare all our results combined on population scale, we find no correlation between ROS burst or phytohormone production with the quantitative resistance observed in phenotyping when we look at each plant individually.

These findings strongly hint that ROS burst or ethylene accumulation as a single immunity component cannot explain the quantitative resistance in *S. chilense* and possible compensatory mechanisms are in place in each plant.

Currently, we are expanding our screenings, looking into other phytohormones and physical barriers like cutin. Ultimately we want to combine all the measures and develop a model to explain quantitative resistance in *S. chilense*.

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Multiplex Real-Time PCR for detection of *Diaporthe*/*Phomopsis* Complex (DPC) species on soybean

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Phomopsis seed decay has been known as one of the most destructive soybean diseases, affecting seed quality and causing yield losses worldwide. The disease is caused primarily by *Diaporthe longicolla* along with other DPC species. Precise identification of the species of this complex is necessary for understanding the epidemiology of the disease and to develop better control strategies. Based on the isolation of 32 DPC strains from DPC-damaged European soybean seeds we identified four different DPC species: *D*. *longicolla*, *D*. *caulivora*, *D*. *eres* and *D*. *novem*. These four species can now be considered the principal DPC species on soybean in Central Europe. We now aim to develop a fast and accurate method to detect these pathogens via multiplex Real-Time PCR. Based on sequences of the international transcribed spacers (*ITS*) and translation elongation factor 1-alpha (*TEF1*), four specific TaqMan primer/probe sets were designed. The specificity and efficiency of the designed primer/probe sets were tested using DNA from pure cultures of these four species and other important soybean pathogens including *Sclerotinia sclerotiorum, Colletotrichum truncatum, Fusarium tricintum*. Our results indicate that these primer/probe sets allow excellent discrimination of the different species and can be used to detect and distinguish these four European DPC species together in parallel using multiplex Real-Time PCR. The multiplex assay has been tested on different plant material including healthy and infected soybean seeds or seed coats, soybean stems, and leaves. Especially results for soybean seeds are highly reliable making our assay a promising new standard procedure for testing soybean seeds.

Iron homeostasis controls cell wall integrity of the maize pathogen *Colletotrichum graminicola*

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Iron is an essential microelement for all organisms. Due to its low solubility combined with the potential to produce highly damaging reactive oxygen species, a tight regulation of iron uptake and storage is essential for all living cells. Pathogenic fungi employ distinct strategies for high affinity iron uptake from the host tissue, i.e. reductive iron assimilation (RIA) and siderophore-

mediated Fe^{3+} acquisition (SIA). Saprophytic hyphal growth under iron starvation leads to an up-regulation of both RIA and SIA pathways. During the biotrophic pathogenic development, RIA is highly active, and SIA is suppressed. The subsequent necrotrophic stage is characterized by efficient killing of the invaded host tissue and a reversal in the iron uptake, with strong activation of SIA and decreasing RIA activity. Maize leaves pre-treated with the siderophore Coprogen showed strongly increased defense responses, including respiratory burst, to *C. graminicola* infection. The role of coprogen as a defense-priming agent may fully explain repression of SIA in biotrophic hyphae of *C. graminicola*. This strategy resembles the specific repression of branched β-glucan synthesis, a strong pathogen associated molecular pattern (PAMP), during biotrophy.

In model fungi such as *Aspergillus nidulans* or *Aspergillus fumigatus*, tight regulation of SIA und RIA occurs on transcriptional level, mediated by the transcription factors SreA and HapX, respectively. However, *Aspergillus* spp. do not infect plants and do neither exhibit biotrophic nor necrotrophic lifestyles. Here we report on the identification of *sreA* and *hapX* homologs of the hemibiotroph *C. graminicola* that were denominated as *CgSRE1* and *CgHAP10*. We showed that both genes are iron-dependently regulated on the transcriptional level in saprophytic hyphae. Targeted deletions of these loci led to delayed growth under iron-limiting conditions. Remarkably, a strong phenotype displayed by hyphae of Δ*sre1* strains is a hyphal integrity defect reported in chitin synthase-deficient mutants and visualized as the formation of intrahyphal hyphae. These clear morphological defects strongly suggest that iron homeostasis controls cell wall-integrity in *C. graminicola*.

Detailed functional characterization of the putative transcription factors CgSre1 and CgHap10 during biotrophic and necrotrophic stages will gain further understanding of iron acquisition and the link between iron and hyphal integrity.

Charakterisierung der *Ht*-Resistenzgene in Mais gegen *Exserohilum turcicum*

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Turcicum-Blattdürre, eine der wichtigsten Krankheiten im Maisanbau weltweit, wird durch das Pathogen *Exserohilum turcicum* verursacht. Die typischen Symptome sind längliche, gräuliche Läsionen im Blatt, die sich im Verlauf der Zeit zur Blattdürre entwickeln. Einer der möglichen Kontrollmethoden gegen Turcicum-Blattdürre ist Sortenresistenz. Bekannte Monogene Resistenzen *Ht1, Ht2, Ht3* und *Htn1* wurden in Isogene Linien eingekreuzt (Welz und Geiger, 2000, Galiano und Miedaner 2017). Das Ziel dieser Arbeit ist die Charakterisierung des Mechanismus und der Wirkung der Ht-Resistenzgene in Mais gegen *Exserohilum turcicum.*

Hierzu wurden kompatible und inkompatible Interaktionen zwischen Rasseisolaten und Maisgenotypen mit den unterschiedlichen Resistenzgenen miteinander verglichen. Das Differentialsortiment besteht aus isogenen Maislinien basierend auf der Referenzlinie B37 mit und ohne die Resistenzgene *Ht1, Ht2, Ht3* und *Htn1*. Die Rassenbestimmung der Isolate erfolgte in vorherigen Untersuchungen (Hanekamp, 2016). Davon ausgehend wurde das Differentialsortiment mit virulenten und avirulenten Isolaten inokuliert und die folgenden Parameter untersucht: Phänotyp, Befallsstärke, Gehalt an pilzlicher DNA, Sporenbildung, Wasserstoffperoxidbildung und histologische Untersuchung der Besiedlung des Blattgewebes. Die Resistenzsymptome der inkompatiblen Interaktionen unterschieden sich zwischen den unterschiedlichen Resistenzgenen. Im Vergleich zur Kontrolle ohne Resistenzgen zeigten B37*Ht2*; B37*Ht3* und B37*Htn* niedrigere Befallstärken und DNAMengen 10 und 14 Tage nach der Inokulation (dpi) für beide Interaktionstypen. Die B37*Ht1* zeigte höheren Befall, obwohl die DNAMenge nicht höher war als in der Kontrolle. Bei B37*Ht1* ist die Nekrose eine schwere Abwehrreaktion des Wirts. Darüber hinaus war die relative Sporulation bei allen inkompatiblen Interaktionen niedriger, außer bei B37*Ht2*. Histologische Untersuchungen mit DAB und NBT Färbungen zeigten (Hückelhoven et al. 2000), dass keine Korrelation zwischen Wasserstoffperoxidbildung und Virulenz bestand. Infolgedessen spielen reaktive Sauerstoffspezies (ROS) keine Rolle bei der Resistenz gegenüber *E. turcicum*. Bei der histologischen Untersuchung der Pathogenese mit Chlorazol Black E Färbung (Brundrett et al. 1984) konnte festgestellt werden, dass die Hyphen grundsätzlich ins Xylem eindringen und bei der kompatiblen Interaktion das Mesophyll stärker besiedelt wird (Kotze et al. 2019). Die Wirkung der monogenen Resistenzgene *Ht1, H2, Ht3* und *Htn1* zeigt sich erst ab 10 dpi. Weitere Untersuchungen zu den Resistenzmechanismen gegenüber *E. turcicum* sind vorgesehen.

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Identification and characterization of Nep1-like proteins from the grapevine downy mildew pathogen *Plasmopara viticola*

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Grapevine downy mildew, evoked by the obligate biotrophic oomycete *Plasmopara viticola*, is one of the most important diseases of viticulture in humid climates. Strongly adapted to its host plant, *P. viticola* achieves infection by suppression or circumvention of plant innate immunity by secretion of effector molecules. One family of potential effectors are „necrosis and ethylene inducing peptide 1 (Nep1) – like proteins (NLPs) “.

NLPs are generally divided into two groups by their ability to induce necrosis. Cytotoxic NLPs are often expressed at a later stage of infection, for example during the switch from biotrophic to necrotrophic lifestyle in hemibiotrophic plant pathogens. These proteins usually represent important virulence factors for their hosts. Beside these cytotoxic NLPs many non-cytotoxic NLPs exist in hemibiotrophic or biotrophic microorganisms. The function of these non-cytotoxic proteins is so far unknown.

During this study eight independent NLPs were identified in the genome of *P. viticola*. Functional analysis of three selected NLPs revealed that none of the putative necrosis elicitors was able to actually induce necrosis, neither in several susceptible or resistant *Vitis* species nor in the dicot model plant *Nicotiana benthamiana*. This inability existed independently of the presence or absence of an N-terminal signal peptide. Interestingly, expression analysis of a presumed pseudo gene revealed remarkable differences between pure sporangia solution and sporangia in the presence of leaf material. This regulation suggests an important function of a so far supposed nonfunctional “pseudo” NLP gene during the first hours of infection.

***In vino veritas* - uncovering the secrets of success of the Asian grapevine leaf rust *Phakopsora euvitis* on grapevine**

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Asian soybean rust (*Phakopsora pachyrhizi*) is known as an aggressive plant pathogen on soybean and is responsible for considerable economic damage. Similarly, Asian grapevine leaf rust (*Phakopsora euvitis* = syn. *Phakopsora meliosmae-myrianthae*) can cause severe rust

epidemics on the perennial plant grapevine. While our understanding of Asian soybean rust constantly increased in the last years and sequencing of the complex genome is expected to further boost this process, there is a need to deepen our knowledge of Asian grapevine leaf rust. In the frame of the project "ProCroPs" (protection of *crop* plants against *Phakopsora* spec.), we aim at a better understanding of the similarities and differences in the biology of both Phakopsora spp. by combining histopathological examinations, comparative growth kinetics, and next-generation sequencing. Comparative genome analysis between both Phakopsora species and the bean rust fungus *Uromyces appendiculatus* uncovered genome size and degree of heterozygosity in the dikaryotic stage of these rusts. We present novel data on Asian grapevine rust *de novo* assembled transcriptome and how this knowledge can be used to increase our understanding of the infection strategy of *P. euvitis*.

SmartBioS: Smarte Biostimulantien für einen kupferreduzierten Rebschutz

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One of the most severe pathogens in organic viticulture is *Plasmopara viticola* causing grapevine downy mildew which shows high impact on overall yield and wine quality worldwide. In sustainable viticulture, P. viticola is controlled by fungicides, primarily based on copper compounds. A synergistic combination of biological and biochemical stimulants presents a promising approach for the protection of vines. Hence, biostimulants such as Trichoderma and chitosan are able to promote plant growth and development. Thereby, they represent a supporting tool in strengthening plant tolerance to abiotic and biotic stresses. For a simultaneous and effective combination strategy of Trichoderma, chitosan and copper fungicides, the compatibility of all agents has to be ensured. Thus, copper tolerance needs to be increased in Trichoderma. Additionally, chitosan serves as a reducing agent for effective copper dosage. Also, Trichoderma releases chitosanolytic enzymes which enables a combination of antimicrobial chitosans. 6 copper tolerant Trichoderma candidates were identified out of 148 isolates belonging to 3 different species. The interaction of Trichoderma and copper ingredients was analyzed microscopically to investigate fungal copper tolerance mechanisms. Additionally, chitosan compatibility was tested with 3 chitosans for all candidates by mycelial growth and spore germination assays. To identify antagonistic potential of Trichoderma and chitosan, leaf disc assays were conducted in vitro. The application of biostimulants showed an inhibition of the pathogenic development. This indicates a potential systemic defense effect triggered by biostimulants treatment against P. viticola. For Trichoderma treatments, this seems to be based on a potential systemic defense effect. As for chitosan treatments, the observed defense effect seems to have both, a triggered systemic and also direct inhibitory cause depending on chitosan used.

Proof of concept for visual rating of Cercospora leaf spot using multispectral UAV image

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Visual rating of Cercospora leaf spot (CLS) is a time-consuming process that demands trained personal. Disease incidence (DI) and disease severity (DS) are the most reliable parameters and tools to identify resistant varieties in the plant breeding process. In 2019 within the context of the COBRI-Project "Sensing of plant diseases by hyperspectral imaging and UAVs ", a block design sugar beet field trial located near Göttingen and inoculated with *Cercospora beticola* was monitored with an unmanned aerial vehicle (UAV) and multispectral camera system in time series. Flight mission was established to acquire images with a ground sample distance (GSD) of 0.4 cm allowing the detection of single leaf spots on raw images. In parallel, ground truth data on DI and DS were assessed to validate digital scoring.

The strategy to analyze orthomosaic multispectral images of inoculated plots was divided into three stages: (1) optimization of spectral parameters to detect Cercospora symptoms, (2) location of plant center and center of symptoms, (3) pixels counting, clustering, distance determination and paring of centers to calculate DI and DS (Figure 1). Results show that Cercospora symptoms can be segmented in multispectral images using the R672/708 index with a threshold value of 0.35. Clustering and centroid calculation of a sugar-beet segmented binary mask at BBCH 15 can allow the location of the plant center. DS is possible to be calculated with the number of plants and by two-mask analysis considering spots and background. DI was determinate with a distance analysis and pairing of plant and symptoms centers is necessary taking into account the digital elevation model of the scene. To date, the complete procedure is being validated in time-series for the complete CLS trial field in a total of 24 plots by using visual rating values of DI and DS. Besides, environmental conditions will be considered including the influence of shadows and the implementation of radiometric calibration.

Rassenbestimmung lokaler Populationen des Erregers der Rapswurzelhals und -stängelfäule, *Leptosphaeria maculans*

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Leptosphaeria maculans (anamorph *Phoma lingam*) verursacht die Wurzelhals- und Stängelfäule an Raps und stellt eine der wichtigsten pilzlichen Krankheiten dieser Kulturart in Deutschland dar. Die schnelle Anpassung von *L. maculans* (LM) an im Anbau befindliche monogene Resistenzen kann zu schnellen Resistenzverlusten führen und erfordert somit eine durchgehende Bobachtung lokaler Rassenspektren des Pilzes.

Das Ziel dieser Untersuchung ist die Beschreibung der LM-Rassenspektren in vier Regionen Deutschlands. Die Typisierung erfolgt an einem Sortiment verschiedener *B. napus*

Differentiallinien, die jeweils die Major-Resistenzgene *Rlm*1, *Rlm*2, *Rlm*3, *Rlm*4, *Rlm*7, *Rlm*9, *Lep*R1, *Lep*R2 und *Lep*R3 tragen. Hierzu werden Keimblätter der verschiedenen Differentiallinien mit einer Sporensuspension des Pilzes inokuliert und 14 Tage nach der Inokulation bonitiert. Die Isolate werden als virulent oder avirulent auf der jeweiligen Differentiallinie eingestuft: Die Einstufung der Isolate basiert auf den Parametern Läsionsgröße, -farbe sowie der Sporenbildung (IMASCORE-Bonitурschema). Zusätzlich wurden genspezifische PCR-Assays für den Nachweis von Avirulenzgenen etabliert, für die zum Teil keine Differentiallinien zur Verfügung stehen. Auf dieser Grundlage konnten in den vier Regionen zwanzig Rassen nachgewiesen werden. Die zwei dominierenden Rassen sind (i) *Avrlm7/Avrlep1/Avrlep2* beziehungsweise (ii) *Avrlm7/Avrlep1*. Die Resultate zeigen, dass einige Resistenzgene wie *Rlm1, Rlm3* und *Rlm4* noch eine partielle Wirksamkeit aufweisen, während *Rlm*2 und *Rlm*9 komplett unwirksam sind. Zurzeit können die Majorresistenzen *Rlm*7 und *Lep*R1 als die wirksamsten Majorgene klassifiziert werden. Dennoch steigt in manchen Regionen die Häufigkeit der Isolate, die Virulenz an *Rlm*7 zeigen. Während in früheren Studien die Anteile um 1% betrugen konnten in dieser Studie an bestimmten Standorten Nachweishäufigkeiten von mehr als 20% festgestellt werden. Derartige Studien können einen Beitrag zum Resistenzgenmanagement leisten. Hierzu wäre allerdings die Bereitstellung von Daten zur Resistenzgenausstattung aktueller Rapssorten notwendig, welche zurzeit nicht verfügbar sind und wohl auf absehbare Zeit nicht zur Verfügung stehen werden.

Trichoderma harzianum -Ein neues Pathogen im Mais?

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Trichoderma harzianum gehört zu der Gruppe der Ascomyceten und ist ein weltweit ubiquitär auftretender Artenkomplex. Aufgrund seiner mycoparasitären und endophytischen Eigenschaft wird es unter anderem in der Landwirtschaft als biologisches Pflanzenschutz- und Pflanzenstärkungsmittel genutzt. Bisher ist diese Art nicht als phytopathologischer Erreger an Maiskolben beschrieben. Im Jahr 2018 konnte jedoch erstmalig an einem Feldversuchsstandorten in Süddeutschland ein massives Auftreten von Trichoderma sp. am Maiskolben beobachtet werden. Aus den befallenen Maiskolben konnte Trichoderma harzianum isoliert und kultiviert werden. Erste Inokulationsversuche unter kontrollierten Bestimmungen im Gewächshaus konnten einen Befall am Maiskolben bestätigen. Dabei zeigten sich, weißes Myzelwachstum zwischen den Körnern sowie massive Produktion von grün- bis graugrünen Konidien auf den Körnern und zwischen den Lieschblättern. Die Befallsstärke wurde visuell anhand der prozentual befallen Fläche am Kolben bestimmt. Dabei zeigten sich vier Isolate von den Standorten Künzing, Pocking, Coix de Pardies (F) und Bernburg als hoch aggressiv und konnten einen Befall von 95-100% generieren. 16 weitere T. harzianum Isolate aus dem süd- sowie mitteldeutschen Raum konnten keinen Befall generieren. Des Weiteren reduzierte sich der Trockensubstanzgehalt, nach der Inokulation mit den

pathogenen Isolaten, signifikant um 30% im Vergleich zur Kontrolle. Mittels weiterer molekulargenetischer Untersuchungen sowie Sequenzierung des Tef 1α Gens konnten diese pathogenen Isolate der Arten T. afroharzianum sowie T. harzianum zugeordnet werden.

Drought stress affects Ramularia leaf spot disease in an assortment of climate adapted barley genotypes

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Um der Anfälligkeit der Landwirtschaft gegenüber dem Klimawandel zu begegnen, wird eine Anpassung von Kulturpflanzen gegenüber extremeren Umweltbedingungen und einem sich wandelndem Krankheitsdruck zunehmend wichtiger (Chakraborty & Newton, 2011). Die Ramularia-Sprenkelkrankheit der Gerste (auch RLS: Ramularia leaf spot) wird durch den pilzlichen Erreger Ramularia collo-cygni verursacht und wird in seiner Ausprägung stark von Klimafaktoren beeinflusst (Havis et al., 2015). Die Krankheit steht daher modellhaft für die Erforschung und das Verständnis der Interaktion von Krankheiten mit Umweltbedingungen. An diesem Schnittpunkt fehlen jedoch Erkenntnisse zur Resistenz gegenüber kombiniertem Klima- und Pathogenstress. Eine starke Sonneneinstrahlung und bspw. Trocken- und Hitzestress tragen zur Ausprägung von sowohl krankheitsbedingten Ramularia- (RLS) als auch den optisch ähnlichen aber physiologisch bedingten Blattflecken (PLS: Physiological leaf spot) während der Kornfüllungs- und Reifephase bei. Diese Entkopplung von Befall, Symptomatik und Epidemie machen die Diagnose und eine gezielte Kontrolle durch richtig terminierte Pflanzenschutzanwendungen sehr schwierig. Erschwerend kommen multiple Fungizidresistenzen von Ramularia collo-cygni gegenüber gängigen Wirkstoffen (Rehfuß et al., 2019). und das Auslaufen der Zulassung des Ramularia-wirksamen Fungizids Chlorthalonil hinzu. Der alternative Anbau Ramularia-resistenter Sorten ist durch eine wenig verstandene und kaum bearbeitete Ramularia-Resistenz in Gerste erschwert, jedoch von zunehmender Wichtigkeit. Die direkten Folgen könnten eine Kontrolllücke und letztlich Ertrags- und Qualitätseinbußen sein. Um im Speziellen den Einfluss von Trockenheit auf verschiedene Gerstensorten und den Krankheitsbefall unter Feldbedingungen zu untersuchen, wurde ein bewegliches Rollgewächshaus („Rain out shelter“) der Bayerischen Landesanstalt für Landwirtschaft am Standort Freising genutzt. Zur Erfassung des natürlichen Befalls mit Ramularia collo-cygni wurden drei Diagnosewerkzeuge angewendet: Erfassung der Symptomatik am Blatt, Sporulation und der spezifische Nachweis der pilzlichen DNA in den Blättern mittels quantitativer PCR. Die Ergebnisse aus drei Versuchsjahren zeigen ein stark verändertes Auftreten von Ramularia-Blattflecken unter trockenen Umweltbedingungen. In den trockengestressten Beständen konnte eine insgesamt geringere Symptomatik, Erreger-Sporulation und niedrigere DNS-Gehalte festgestellt werden. Die Mehrheit der Sorten wurde unter Trockenstress kaum befallen, wohingegen die Trockenheit auf einzelne Sorten nur einen geringen Einfluss hatte. Eine generell hohe Differenzierung im Befall konnte dagegen in den bewässerten Parzellen festgestellt werden. Die gewonnenen Erkenntnisse zeigen innerhalb des getesteten Sortiments starke Unterschiede in der Resistenz gegenüber Ramularia collo-

cygni sowohl unter kontrolliert feuchten als auch unter extremen Trockenstressbedingungen. Die Ergebnisse lassen Rückschlüsse auf die Entwicklung der Ramularia-Sprenkelkrankheit unter sich verändernden klimatischen Bedingungen zu und zeigen Potentiale von einigen im Anbau verwendeten Sorten und aktuellem Zuchtmaterial für eine gezielte Ramularia-Resistenzzüchtung.

Biochemical characterization of MLO2 in *Arabidopsis thaliana*

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Mildew Resistance Locus O (MLO) proteins are plant-specific seven transmembrane domain proteins that are conserved among monocots and dicots. Loss of function of Mlo in *Hordeum vulgare* or loss of function of MLO2 in *Arabidopsis thaliana* provides durable resistance against powdery mildew fungi. Until now the biochemical function(s) of MLO proteins remain(s) unknown. To gain more insights into its biochemical function(s), we use different MS-based strategies to identify new protein interaction partners of MLO2 in *Arabidopsis thaliana*. One of these strategies involves the *in vivo* crosslinking of putative MLO2 complexes followed by tandem affinity chromatography and the identification of putative interaction partners by mass spectrometry. In addition to the known interaction partner of MLO2, calmodulin, different putative *in vivo* protein interaction partners with a link to plant defense responses were identified. Moreover, phosphopeptides of MLO2 have also been recorded by mass spectrometry. Therefore, the phosphorylation of MLO2 by different pathogen-induced kinases, e.g. mitogen-activated protein kinases and calcium-dependent protein kinases was confirmed by a non-radioactive *in vitro* kinase assay. Additionally, we show that the phosphorylation of *Hv*Mlo might influence its biological activity in transient overexpression experiments in the barley *mlo* mutant using different phospho-variants of *Hv*Mlo. Taken together, we could identify new *in vivo* protein interaction partners of MLO2 and we show that phosphorylation might regulate the biological activity of MLO proteins.

Nematode ascaroside ascr#18 primes plants for enhanced defense

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Nematodes are ubiquitous plant parasites causing more than 150-billion-dollar crop losses per year. However, when adequately recognized, nematodes can also activate effective plant defense responses and disease resistance. Recognition of nematode presence by plants frequently occurs by detection of ascarosides, which are evolutionary conserved compounds with a role in nematode development. In plant-pathogenic nematodes ascr#18 is the most abundant ascaroside. The compound appears to be highly active at inducing defense priming and disease resistance in some plants. We show that pretreatment with ascr#18 conditions parsley cells for enhanced Pep13-induced secretion of furanocoumarin phytoalexins and primes Arabidopsis for augmented activation of defense genes upon Pseudomonas syringae pv. tomato DC3000 challenge. To disclose the molecular mechanism by which ascr#18 primes plant defense, we used formaldehyde-assisted isolation of regulatory DNA elements (FAIRE) to show that treatment of Arabidopsis plants with ascr#18 extrudes nucleosomes from the 5' regulatory region of selected defense-related genes. The eviction of nucleosomes is associated with the formation of open chromatin in the 5' regulatory region of genes and with enhanced capacity of genes to be expressed. Together, our results disclose that ascr#18 can prime plant defense by modification of chromatin the promoter region of defense genes, associated with chromatin opening and enhanced gene expression upon challenge. Thus, ascr#18 and possibly other ascarosides may have potential for future sustainable crop protection.

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Advanced CRIPR/Cas technology for fungicide resistance research

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Botrytis cinerea is a major plant pathogen, causing enormous pre- and postharvest crop crop losses. Control by fungicides has become increasingly difficult due to the worldwide apperance of resistance. For example, *B. cinereal* strains simultaneously resistant to five or more classes of fungicides have been found in strawberry fields in Germany and Southeastern U.S.A., which seriously reduce fungicide efficacy.

We report on the establishment of the CRISPR/Cas9 technology for *B. cinerea* and its application for fungicide research. Introduction of Cas9-sgRNAs ribonucleoprotein (RNP) complexes into protoplasts allows highly efficient genome editing. A novel strategy was

established for marker free co-editing, based on cotransformation of a non-integrative telomere vector for transient selection and a non-selected CRISPR/Cas editing construct, resulting in up to thousands of edited transformants. We demonstrate the performance of this strategy by random mutagenesis of codon 272 of the *sdhB* gene, a major determinant of resistance to succinate dehydrogenase inhibitor (SDHI) fungicides, by in bulk-replacement with codons encoding all 20 amino acids. As shown by deep sequencing, all exchanges occured at similar frequencies in the absence of selection, indicating little effects of these exchanges on fungal viability, whereas SDHI selection allowed the identification of several novel amino acid substitutions which conferred differential resistance levels towards boscalid, fluopyram and pydiflumetofen. Similar studies are possible now with any other fungicide target, in the target organism, to characterize binding pockets of active ingredients, test binding hypotheses and their biological effects, which is expected to accelerate rational drug design.

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Introducing *Arabidopsis thaliana* as a host for *Cercospora beticola*

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Due to its high damaging potential, leaf spot disease caused by *Cercospora beticola* is a continuous threat to sugar beet production worldwide. Numerous investigations have been done on disease development and symptomology but only little is known about the early infection stages such as epicuticular growth, penetration or colonization of the tissue. Although there are some reports about the suppression of gene expression in sugar beets, molecular studies are still limited due to lack of mutant plants or databases.

To fill theses gaps, we introduce the model plant *Arabidopsis thaliana* as a host *for Cercospora beticola*. Beside advantages like small size and rapid growth, the Arabidopsis genome is completely sequenced, enabling molecular research. In addition, *A. thaliana* has already been used to study many plant-pathogen interactions including pathosystems with the sugar beet cyst nematode *Heterodera schachtii*, or with necrotrophic fungi like *Altanaria brassicola*. The aim of the work was to establish the *C. beticola – A. thaliana* pathosystem to have opportunities to explore the molecular modifications in the host plant depending on the infection stage. Therefore, we performed infection assays and microscopic observations, that showed that the pathogen can complete its lifecycle (penetration, colonization, sporulation) on *A. thaliana* under greenhouse and sterile conditions.

Identification of unrelated powdery mildew avirulence effectors and their surveillance by allelic barley Mildew Locus A immune receptor.

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Disease resistance is mediated by recognition of pathogen avriulence effectors (AVR) by host nucleotide-binding leucine-rich repeat receptors (NLR). The barley (*Hordeum vulgare*) Mildew locus A (Mla) encoded NLRs (MLAs) confer isolate-specific resistance to the widespread mildew fungus *Blumeria graminis* forma specialis *hordei* (Bgh) and some unrelated fungal phytopathogens. Molecularly characterised MLA NLR variants disclosed that Mla has been subjected to extensive functional diversification at the encoded LRR domains, resulting in allelic resistance specificities, each recognizing a cognate Bgh AVRa. A high-resolution genetic association approach identified the Bgh avirulence loci AVRa1, AVRa7, AVRa9, AVRa10, AVRa13 and AVRa22, all of which encode small secreted proteins. The AVRA effectors are seemingly unrelated, except allelic AVRA10 and AVRA22 that form a balanced polymorphism in the pathogen population. We connect AVRa genomic location with previous linkage studies and show the ability of AVRAs to induce activation of matching MLA variants in monocots and dicots, thereby defining respective AVRA proteins as one major determinant for differential MLA controlled infection phenotypes on barley. Our data revealed and defined the interaction of AVRA proteins with cognate, allelic MLA receptors in planta and in yeast, despite the lack of any sequence and predicted structural similarities between most of the identified AVRA effectors. As such, diversification of Mla is not a result of the evolutionary pressure imposed on the NLRs by direct recognition of sequence-related pathogen effectors, but likely encoded by the AVRA virulence activities in the barley host and these activities can now be determined.

Elucidating the molecular basis of quantitative disease resistance in the maize – *Ustilago maydis* interaction

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The biotrophic pathogen *Ustilago maydis* causes smut disease on maize and induces the formation of tumours on all aerial parts of the plant. Unlike in other biotrophic interactions,

no gene-for-gene interactions have been identified in the maize – *U. maydis* pathosystem. Thus, resistance of maize to *U. maydis* is considered a polygenic, quantitative trait. Here, we aim to elucidate the molecular mechanisms of quantitative disease resistance in maize and how *U. maydis* interferes with its components.

Based on quantitative scoring of disease symptoms in 26 maize varieties, we performed an RNA-Seq analysis of six *U. maydis*-infected maize lines of highly diverging resistance levels. This identified 434 *U. maydis* genes being differentially expressed between maize lines, of which 76 are predicted to encode effectors. We generated *U. maydis* CRISPR/Cas9-KO mutants for selected candidate effector sets. Infections of different maize lines with the generated mutants show that five of these effectors have a maize line-specific virulence function.

On the host side, 3681 genes were differentially regulated in response to *U. maydis* infection across maize lines. Enrichment analysis for Gene Ontology shows that these genes are enriched for extracellular genes as well as for genes associated with protein phosphorylation and oxidation/oxidative stress response, which both are key processes in plant defence.

Taken together, this study not only shows that *U. maydis* effectors have a maize line-specific contribution to virulence, but also reveals that host responses to the infection are dependent on the maize line. Our functional characterization of the maize line-specific effectors and identification of their respective host targets will provide new insights into the molecular mechanisms underlying the maize – *U. maydis* interaction.

Structural and functional analysis of LORE-dependent 3-hydroxy fatty acid immune sensing in Brassicaceae

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The LRR receptor kinase MIK2 mediates pattern-triggered immunity responses against *Fusarium* species in Arabidopsis.

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Fusarium is a genus of fungi causing severe economic damage in many crop species exemplified by *Fusarium* Head Blight of wheat or Panama Disease of banana. Plants sense immunogenic patterns (termed elicitors) at the cell surface contributing to disease

resistance *via* the activation of pattern-triggered immunity (PTI). Knowledge of such elicitors or corresponding plant immunity components is largely lacking for *Fusarium* species. We describe a new peptide elicitor fraction present in several *Fusarium spp.* which elicits canonical PTI responses in *Arabidopsis thaliana* but depends on a currently unknown perception mechanism. We therefore employed a forward-genetics screen using Arabidopsis plants containing a cytosolic calcium reporter (apoaequorin) to isolate *fere* (*Fusarium Elicitor Reduced Elicitation*) mutants. The *fere1* mutant showed impaired PTI marker responses to an enriched elicitor fraction derived from *Fusarium oxysporum* but normal responses to known fungal elicitors. We mapped the causal mutation to the receptor-like kinase MIK2 (MALE DISCOVERER1-INTERACTING RECEPTOR LIKE KINASE 2) with a hitherto undescribed role in PTI pathways but documented functions in other cell-surface signalling pathways. The strong loss-of-function phenotype in various *mik2* alleles to the enriched *F. oxysporum* elicitor and the ability of MIK2 to confer sensitivity to the elicitor in *Nicotiana benthamiana* support that MIK2 is a key component in sensing *Fusarium*. Elicitor responses also partially depend on PTI signalling components known for other cell-surface elicitor responses such as BAK1, BIK1, PBL1, FERONIA, LLG1 and RBOHD. This shows that Arabidopsis senses *Fusarium* by a novel receptor complex at the cell surface that feeds into common PTI pathways and positions MIK2 as a central player that potentially integrates plant endogenous signals with biotic and abiotic stress responses.

B) Abstracts der Poster

(in alphabetischer Reihenfolge nach den Präsentierenden)

Scopoletin provides plant tolerance to different biotic stresses

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The coumarin derivative scopoletin supports the plants defense against both biotic and abiotic stresses, therefore promoting its use in agriculture. In vitro experiments disclosed fungistatic activity of scopoletin against *Phakopsora pachyrhizi* (Pp) associated with reduced accumulation of reactive oxygen species (ROS) in fungal preinfection structures. Non-antioxidant and antioxidant molecules including coumarins with a similar structure to scopoletin were inactive or much less effective at inhibiting fungal accumulation of reactive oxygen species and germination of Pp spores. In a transgenic approach, we aimed to genetically engineer plants with improved stress tolerance. Overexpression of AtF6'H1 -

encoding for the key enzyme in scopoletin biosynthesis - resulted in accumulation of scopoletin and its glycoside scopolin in different plant species. Transgenic soybean plants showed a higher tolerance to Sudden Death Syndrome, likely due to scavenging of mycotoxin induced ROS. Moreover, larvae of *S. exigua* preferably fed on wildtype than on AtF6'H1 overexpressing soybeans. In order to further boost scopoletin biosynthesis and overcome substrate limitations observed in precursor feeding experiments, we co-expressed a transcription factor with AtF6'H1 which globally increased expression of secondary metabolism-associated genes and strongly elevated levels of both scopoletin and its glycoside scopolin.

The Arabidopsis leucine-rich repeat receptor kinase MIK2 is a crucial component of pattern-triggered immunity responses to Fusarium fungi

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Root growth inhibition induced by the immune elicitor flg22 is mediated by cell cycle arrest

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Pattern-triggered immunity (PTI) is highly efficient in protecting plants against the majority of harmful pathogens but is known to negatively interfere with plant growth and to affect crop yield, which can limit breeding efforts to exploit the full resistance and yield potentials in crops. In *Arabidopsis*, flg22 (active flagellin epitope) detection by the receptor FLAGELLIN-SENSING 2 has been key to uncovering PTI regulation and organisation. Here we scrutinise the effects of flg22 on root growth inhibition. We show that flg22 inhibits cell division as a determinant of growth but does not affect stem cell niche function. We find that immunity affects the G2-mitosis transition pathway and stimulating this pathway by over-expressing *CYCLIN-DEPENDENT PROTEIN KINASE B1;1* we re-install regular growth upon full immunity activation. By demonstrating the feasibility of uncoupling negative growth-immunity effects, our findings may inform future sustainable breeding strategies to generate plants with high resistance traits and unimpaired growth.

Identification of histone methyltransferases in defence priming

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Systemic acquired resistance (SAR) is the systemic broad-spectrum resistance of plants after local pathogen attack. SAR as well as detection of microbe-associated molecular patterns, perception of pathogen effectors, or treatment with certain chemicals induce defence priming. This refers to the enhanced capacity of cells to mobilise defence responses. Defence priming has been shown to be associated with covalent modification of histones in the promoter and promoter-proximal region of defence genes, such as the WRKY transcription factor-encoding gene WRKY6. Some of these modifications seem to prime the gene for enhanced transcription upon challenge. Among those modifications, trimethylation of the lysine 4 residue in histone 3 (H3K4me3) has caught much attention because it is proposed to provide an immunological memory (Conrath et al., 2015). To identify the histone methyltransferase(s) that write the H3K4me3 modification during priming we focused on those enzymes containing the conserved SET domain and are described or annotated as H3K4me3 writers. Therefore, we screened Arabidopsis knockout lines for impaired defence priming and reduced SAR. The presented results provide further correlative evidence for the importance of H3K4me3 to defence priming.

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Barley RIC157 – Building a bridge to facilitate RACB-mediated susceptibility to powdery mildew

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Successful pathogens do not only undermine plant defense reactions, they also take advantage of certain host processes. Full susceptibility of barley to penetration by the biotrophic ascomycete *Blumeria graminis* f.sp. *hordei* (*Bgh*) requires the presence of RACB, a small monomeric G-protein (ROP, RHO of plants). RACB has been shown to be involved in cell polarization and cytoskeleton reorganization, however an exact mode-of-action of RACB-

mediated susceptibility remains obscure. Scaffolds like previously described RIC proteins potentially establish links to various downstream targets upon direct interaction with activated ROPs via a highly conserved CRIB motif. Here we describe a yet uncharacterized barley RIC protein, RIC157, that can directly interact with RACB in yeast and in planta. This direct interaction is likely a prerequisite for RIC157 being recruited to the cell periphery and plasma membrane in the presence of activated RACB. We also show a co-localisation of activated RACB and RIC157 at the penetration site, specifically at the haustorial neck, during *Bgh* infection. Moreover, transiently overexpressed RIC157 renders barley epidermal cells more susceptible to *Bgh* in a RACB-dependent manner. Taken together, our data indicate that RIC157 promotes fungal infection into barley epidermal cells by potentially acting as downstream executor in RACB signaling.

Roles of the protein domains of the B-lectin S-Domain receptor kinase LORE in oligomerization and immune signalling

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VitiMeteo - an internet platform for sustainable viticulture

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Forecasting and decision support systems (DSS) are basic tools for integrated and biological plant protection. VitiMeteo (VM) is an extensive and free online accessible DSS for viticulture. It is a long term cooperative project between the State Institute of Viticulture and Enology (WBI, Germany), the Swiss Research Station Agroscope (Switzerland) and the company GEOsens (Germany). Basically, the system consists of data sources, a database, expert software and the presentation of data on the internet. Core of this system is the database Agrometeo (Switzerland), where all data from different weather stations and related forecast data are stored. The first module “VM Plasmopara” was launched in 2002. This model calculates the most important steps of the life cycle of downy mildew (Plasmopara viticola), a major disease in viticulture. Since then VitiMete grew step by step.The next milestones were the integration of the growth model “VM Meteo Growth”, which was programmed in cooperation with H.-R. Schultz (Hochschule Geisenheim University, Germany) and the powdery mildew (Erysiphe necator) risk model “VM Oidiag”, based on work of W. Kast (LVWO

Weinsberg, Germany). Other expert models like “VM Insects”, “VM Hyalesthes” and “VM Rustemite” followed. Practical applications are the calculations of the flight start of the grape berry moth (Paralobesia viteana) and of the cixiid planthopper Hyalesthes obsoletus. “VM Hyalesthes” is built on the works of M. Maixner (JKI Siebeldingen, Germany). The latest models are "VM Black rot”, which calculates the important parts of the life cycle of Black rot (Guignardia bidwellii) and “VM Phenology”, which simulates the phenological stages of the cultivars, Riesling, Pinot noir and Müller-Thurgau. Both softwares were developed together with D. Molitor (Institute of Science and Technology, Luxembourg). "VM Data Graph" is an additional, valuable online tool for the visualization and validation of weather data. Linking weather forecast, provided from the company meteoblue (Switzerland), with all models marked another milestone in the development of the system. Nowadays VM models are widely used in other European countries in, e.g. Austria, France and Switzerland. The "VitiMeteo system" is open, flexible and innovative, because research results and new models can be put into practice more quickly than before. With the help of the VitiMeteo platform winegrowers can better schedule their fungicide or insecticide treatments, which reduce the application of pesticides and thus contribute to a more sustainable viticulture.

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

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Ustilago maydis is a smut fungus that infects all aerial developing tissues of maize. It has been shown that the correct cellular developmental program of the host is a crucial factor to enable pathogenicity. Thus, overexpression of proteins that elicit immunomodulatory or developmental functions are expected to result in milder disease symptoms. Previous studies

indicate the importance of small secreted proteins in immunity and development. Based on proteomic datasets, we select candidate maize proteins and verify secretion in transient tobacco assays. To study the influence of candidate proteins in development and pathogenicity we are using the Trojan horse strategy. This assay exploits the pathogens secretory machinery to deliver novel small secreted proteins into the biotrophic interaction zone. Candidate maize proteins are cloned into *U. maydis* to generate the Trojan horse strains and subsequently infect maize plants. By using this strategy, we already identified three small secreted maize proteins that show a phenotype. 17 maize proteins are under testing at the moment. Current results will be presented.

(Z)-3-Hexenyl acetate, a Volatile Organic Compound, modulates Susceptibility in Barley-Powdery Mildew Interaction

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CRISPR/Cas9 with ribonucleoprotein complexes allows highly efficient marker-free editing in *Botrytis cinerea*

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Botrytis cinerea is is considered one of the most important plant pathogens, causing enormous pre- and postharvest crop crop losses of fruits, vegetables and flowers. We report on the establishment of the CRISPR/Cas9 technology for *B. cinereal*, based on introduction of Cas9-sgRNAs ribonucleoprotein (RNP) complexes into protoplasts. Using *Bos1* as a target gene which allows positive selection of mutations, highly efficient genome cleavage and repair via non-homologous end joining (NHEJ) was obtained, and homologous recombination using repair templates with 60 bp homology flanks resulted in targeted editing with 90% efficiency. Two novel cotransformation strategies were established for marker free editing. Resistance marker shuttling is based on the exchange between different resistance markers in each transformation. An even more effective strategy used cotransformation of a non-integrative telomere vector for transient selection and a non-selected CRISPR/Cas editing construct, resulting in up to thousands of transformants and coediting rates of 10-65%. This approach allowed the marker-free introduction of mutations via NHEJ, GFP-tagging by knock-in of a superoxide dismutase (BcSod1), and deletion of virulence-related proteins. Telomere-

mediated coediting worked also with *Magnaporthe oryzae*. The unprecedented performance and ease of use of these RNP-based tools, which don't require any cloning steps, will greatly improve molecular research with *B. cinerea* and can be transferred to other fungi.

Reference:
Leisen T et al.(2020). CRISPR/Cas with ribonucleoprotein complexes and transiently selected telomere vectors allows highly efficient marker-free and multiple genome editing in *Botrytis cinerea*. *bioRxiv preprint doi:* ***https://doi.org/10.1101/2020.01.20.912576***

Role of the plant vacuole in plant immunity

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The phytohormone salicylic acid (SA) mediates plant defence against pathogens and has been shown to impact on plant growth as well. Previously, we have demonstrated that cell size and vacuolar morphology is eminently linked (Scheuring et al., 2016; Kaiser et al., 2019). Therefore, we investigated how SA treatment impacts on vacuolar morphology and how this in turn affects cell size and root organ growth. In addition, we try to link structural to functional changes, relating altered vacuolar morphology to plant immunity. To do so, we have carried out MS-MS analysis of isolated vacuoles from leaves infected by the necrotrophic fungus *Botrytis cinerea*. Notably, several potential plant-defence related proteins were found accumulating in the vacuole. This underlines the important role of the plant vacuole for plant immunity and could be related to SA-induced changes.

Comparative profiling of defense-associated secondary metabolites in tomato wild species *Solanum chilense*

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Detection and control of *Fusarium graminearum* and *Sclerotinia sclerotiorum* in the new NOcsPS cropping system

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The use of chemical-synthetic plant protection products (csPPP) is seen increasingly critical by cosumers. Additionally, the use of csPPP underlies more and more restrictions. However, their application is necessary to maintain high yield and good quality of crops, that are cultivated in cropping systems with close crop rotation and high intensity. On the other hand, organic agriculture is not able to produce sufficient yield to feed a growing population. Thus, the development of a new cropping system, which allows the targeted use of mineral fertilizer but renounces csPPP, is required. This system is called the NOcsPS cropping system. One requirement for a sustainable realization of the new NOcsPS cropping system is a rapid and precise detection of pathogens in the field. Also, new biological control agents (BCAs) have to be found and established as substitutes for csPPP.

In this study pathogen specific changes in plant metabolism caused by F. graminearum in wheat and S. sclerotiorum in soybean will be detected by a hyperspectral camera in a lab scale. Plants are artificially inoculated and reflectance of ears (wheat) or whole plants (soybean) is measured. In combination with molecular detection methods this allows to establish an early detection of plant pathogens. This monitoring system will also be transferred to the field. Furthermore, several new BCAs will be characterized and tested for their efficacy to control pathogens, both in the greenhouse and at field scale. These BCAs have the potential to replace csPPP in the long term.

The aim of the study is to establish a monitoring system using a drone-based hyperspectral measurement setup. By flying over entire wheat and soybean fields this allows the detection of pathogens as well as the examination of efficacy of several fungal and bacterial BCAs to control F. graminearum and S. sclerotiorum.

Generation and functional characterization of artificial effector gene clusters in *Ustilago maydis*

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Smut fungi constitute the second largest group of plant pathogens subsequent to the rusts fungi. The members of this group affect grasses of the *Poaceae* family, including economically

relevant crops worldwide. Most smut fungi colonize their host plants systemically and produce symptoms in the inflorescences, where teliospores are formed. A peculiar exception is the smut fungus *Ustilago maydis*. This pathogen presents a biotrophic lifestyle, being able to produce tumors in all aerial parts of its host maize trough organ-specific effectors.
U. maydis has a compact genome, is amenable to reverse genetics and adaptable to grow under laboratory conditions. These traits and the well establish plant-pathogen system, make it a model organism to understand the biology of functional effectors and bio-trophic fungal lifestyle.

Secreted effector proteins play a vital role during colonization and tumor formation. Parasitic interactions evolve toward the variation of effector repertoire. This evolution is caused by alterations between the pathogen population to overcome plant immune responses. Based on our current knowledge on effector function and evolution, we propose a model, which classifies effectors in functional categories that correlate with co-evolution of pathogen and host plants.

To functionally study effector genes and their transcriptional regulation in *Ustilago maydis* and its closely related maize pathogen *Sporisorium reilianum*, we are generating a tool box with artificial gene modules. These genetic constructs represent different parts of genes as independent entities, able to be combined unrestricted between them from the same or different species, allowing the formation tailor-made gene clusters which allow the temporally and spatially defined expression of individual effector genes. This approach aims to define the role of effectors at specific stages of infection, as well as to understand how transcriptional regulation of effectors defines their function in virulence.

A long term vision of this approach is to understand the biology of obligate pathogens not amenable to reverse genetics by combining modules well establish in *Ustilago maydis* with unknown effectors of organisms such as the bio-trophic rust fungi.

Rainfast release systems for efficient copper-based crop protection

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Approximately 40% of all crops would be lost to plant pathogens, insect pests and weeds without application of appropriate pesticides. However, reducing pesticide use is of high importance for a sustainable agriculture because plant protection agents pose a potential risk to the environment and consumers. One of the major drawbacks of currently used pesticides is their insufficient rainfastness. Exposure of plant surfaces to rain and irrigation causes pesticide wash off and thus reduced plant protection. The BioSC focuslab *green*Release developed a novel technology platform for improved disease management which is based on the immobilization of microgel containers. We tested microgel containers with attached anchor peptides and incorporated active ingredients, i.e. Cu^{2+} ions. Microgels that were applied to the surface of apple and sugar beet leaves distributed evenly in a homogenous layer. Due to the incorporation of biological active copper ions the symptom formation of the pathogens *Venturia inaequalis* (causing agent of Apple Scab) and *Cercospora beticola* (causing agent of Leaf Spot Disease) was nearly abolished; even at very low concentrations (27 µg/ml active ingredient). Moreover, this protection retained on inoculated sugar beet plants, even after the simulation of 7 mm rainfall. By testing microgels loaded with active ingredients, we could once again demonstrate the effectiveness of the technology in terms of fungicidal activity, plant protection and rainfastness.

Mechanisms of antagonism in the leaf microbial community of *Arabidopsis thaliana*

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Plant organs are largely colonized by different microbes which interact with each other forming a community. Sampling *Arabidopsis thaliana* leaves revealed certain microbial hubs (Agler et al., 2016) which play an important role in the community structure. One example is *Moesziomyces* sp., a basidiomycete yeast which was found to reduce *Albugo laibachii* leaf infections in A. thaliana. RNA seq analysis identified candidate genes in *Moesziomyces* sp. being upregulated in presence of *A. laibachii*. Among these, *Moesziomyces* secretes a member of the Glycoside hydrolase (lysozyme) family 25, which has a strong impact on *A. laibachii* fitness. The lysozyme produced heterologously in *Pichia pastoris* was tested for its enzymatic activity by turbidimetric assay and agar diffusion test with *Micrococcus lysodeikticus*. We are testing the lysozyme activity on *A. laibachii* and members of the bacterial community on *A. thaliana* leaves to elucidate the mechanism of this microbial antagonism.

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Verticillium longisporum suppresses plant defensin PDF2.2 to initiate a compatible plant-fungus interaction in _Arabidopsis thaliana_

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In both of oilseed rape (Brassica napus) and Arabidopsis plants, knockout of the gene CRT1a encoding Calreticulin, one of putative plant compatibility factors, results in plant resistance to the Verticillium longisporum infection, which is however accompanied with an enhanced expression of PDF2.2 in plants. To understand the underlying mechanism, we generated PDF2.2-overexpression and -knockdown Arabidopsis lines and challenged these with the fungus. We observed that the knockdown of PDF2.2 highly enhanced plant susceptibility to the fungal infection with pronounced fungal colonization and symptom development. To contrast, the overexpression of PDF2.2 resulted in plant resistance with impaired development of symptoms and fungal colonization. Most Interestingly, in Arabidopsis PDF2.2 is constitutively expressed at a high level in the whole plant, but pronouncedly suppressed at the early infection stages (6dpi) as revealed by its transcript abundance and the GUS expression levels under the control of PDF2.2 promoter. Here, we report that suppression of PDF2.2 belongs to the virulence mechanism of the fungus to initiate the infection process in the host. A possible model is discussed.

The H3K4 methyltransferase gene _KMT2_ is a novel virulence factor of the maize anthracnose pathogen _Colletotrichum graminicola_

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The histone-methyltransferase gene *KMT2* is responsible for mono-, di- and trimethylation of lysine K4 of histone H3 (H3K4). *KMT2* is evolutionarily highly conserved and was first described as *SETI* in *Saccharomyces cerevisiae*. The gene encodes the enzymatically active subunit SetI of a protein complex called Compass. This complex transfers methyl-groups to H3K4 and contributes to the establishment of transcriptionally active euchromatin. Thus, *KMT2* plays an important role in epigenetic transcriptional regulation.
The ascomycete *Colletotrichum graminicola* is a hemibiotrophic plant pathogen, which penetrates and colonizes its host plant through an appressorium and forms primary hypha. Subsequently, secondary hyphae differentiate, necrotize the surrounding host tissue and cause anthracnose disease symptoms. As differentiation of infection structures requires

significant transcriptional re-programming, epigenetic regulatory factors may play a key-role in pathogenic development.
We used homologous recombination to generate Δ*kmt2* mutants of the maize anthracnose fungus *C. graminicola*. Δ*kmt2* mutants showed a severe defect in hyphal growth and asexual conidiation, and conidia were significantly smaller, as compared to those of the wildtype (WT) strain. Infection essays on *Zea mays* clearly showed that Δ*kmt2* had severe defects in appressorial penetration, and were therefore significantly less virulent than the WT strain. Few appressoria were able to penetrate an epidermal host cell, but were subsequently unable to colonize the plant efficiently, due to developmental defects in *in planta* differentiated infection hyphae. In conclusion, we show that H3K4 methylation plays a significant role in pathogenic development of *C. graminicola* and identified *KMT2* as a novel virulence factor.

Using multi-omic resources to learn more about putative roles of orphan receptor-like proteins in *Arabidopsis thaliana*

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Leucine rich-repeat (LRR) recpetor-like proteins (RLPs) have been implicated in a variety of different process, ranging from development to defence against pathogens. Despite having more and more data available the role of most of these RLPs remains still elusive.

We want to use a multi-omic approach using transcriptomic and proteomic data from various tissues combined with the genomic data from the Arabidopsis 1001 Genome project in order to identify potential patterns within the RLPs, linking these patterns to putative functions.

Does histone lysine methylation control host-adapted gene expression in *Sporisorium reilianum*?

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Sporisorium reilianum is a biotrophic smut fungus that exists in two *formae speciales* (ff. spp.) with different host plant preferences, i.e., one causes head smut of sorghum (f. sp. *reilianum*) and the other of maize (f. sp. *zeae)*. Both can infect the non−compatible host but will not produce spores. The gene composition is nearly identical, but the gene expression is highly dependent on the host plant. The excessive change in gene expression without many changes

in DNA sequence may depend on epigenetics. We hypothesize that the difference in gene expression depends on epigenetic regulation through histone methylation. Genome analysis revealed at least three putative histone lysine methyltransferase (HKMT) genes in *S. reilianum*. The three genes were identified as putative *DIM−5*, *SET−1* and *SET−2* homologs, respectively. Genes were deleted in *S. reilianum* f. sp. *zeae* and confirmed by Southern blot. Abnormalities in growth and cell morphology were observed in *srset*−2 and *srdim−5* mutant lines. Both mutant lines showed reduced growth. In addition, *srset−2* mutant showed changes in cell morphology from yeast-like to filamentous and *srdim−5* showed mating inability. In contrast, no effect on growth and morphology was observed in strains lacking *SrSET−1*. Strains lacking *SrSET−1* were able to enter and proliferate inside the leaf tissue but spore formation was reduced compared to the wild type. These results indicate that epigenetic control mechanisms exist in *S. reilianum* and that they control growth and infection-related genes. Further gene expression studies of the HKMT deleted strains grown axenically and in-planta will reveal the expression changes during the infection cycle. ChIP−Seq experiments are planned that will reveal whether effector genes are regulated by the HKMT Set−1.

UAV based hyperspectral imaging combined with modern data analysis for non-invasive disease detection improves efficiency of precision farming

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Despite the recent advances in plant disease detection with optical sensors techniques which have proven successful in research studies are not being employed in agricultural practice due to measurement difficulties of hyperspectral imaging under field conditions and at a sufficient scale for practical applications.

This study presents a combination of close range hyperspectral imaging time series measurements of plant pathogen interactions, which explore the pathogen specific changes in plant metabolism during the disease progression, and drone based multispectral measurements, which enable high throughput disease screenings on field scale. The relevant information in the respective datasets are highlighted through the use of modern data analysis methods – in supervised and unsupervised approaches.

The goal of the study is to fly over entire fields with the drone-based hyperspectral measurement setup and automatically create disease maps, which can be used to precisely initiate plant protection measurements. This project has the potential to increase the efficiency in precision farming.

Analysis of PRONE GEF14 as a potential activator of ROP GTPases in barley powdery mildew

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RAC/ROP GTPases are molecular switches that are crucial in the regulation of plant development and immunity. In domesticated barley (*Hordeum vulgare*), the ROP RACB plays a major role in the interaction with the powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*). For instance, knock-down of *RACB* renders the plant more resistant to pathogen attack whereas mutationally aided activation supports fungal penetration into host epidermis cells. Endogenous activation of GTPases is typically catalysed by their interaction with guanine nucleotide exchange factors (GEFs), by facilitating the exchange of GDP with GTP and thereby turning ROPs into an active signalling state. Plants possess an independent class of GEFs with a plant-specific RAC/ROP nucleotide exchange domain (PRONE). In this work we analyse barley PRONE-GEF14 as a potential RACB activator and component of the RACB-mediated susceptibility pathway. We confirmed direct protein-protein interaction between RACB and PRONE-GEF14 in yeast and *in planta* and aim to assess the ROP activating capability of PRONE-GEF14 by establishing a specific FLIM/FRET sensor.

Lineage characterization and phylogenetic analysis of *V. longisporum* strains from European and Canadian oilseed rape fields

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Verticillium longisporum is a vascular fungal pathogen of cruciferous crops that originated from at least three hybridization events between different *Verticillium* spp., leading to the hybrid lineages A1/D1, A1/D2, and A1/D3. Although A1/D1 is considered the most relevant lineage in oilseed rape, an extensive study with a broad temporal and geographic scope was performed to confirm this hypothesis. In order to assess whether there are genetic subgroups within this lineage that might have agronomic importance, a phylogenetic analysis was conducted based on the output of genotyping by sequencing. To assess the agronomic importance of the candidate isolates, their aggressiveness was tested on oilseed rape under greenhouse conditions. This study confirms that A1/D1 is the prevalent agggresive lineage of *V. longisporum* in European and Canadian oilseed rape fields. Although the genetic clusters within A1/D1 lineage do not illustrate a clear geographic separation, the resulting phylogenetic tree indicates a Swedish/East Europe and French/German origin of the recent disease development in Canada and England. Additionally, *V. longisporum* has a higher genetic

diversity in France and Germany. The study also revealed a high level of variability of the A1/D1 aggressiveness within genetic clusters, countries and locations.

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Investigating the armory of *Magnaporthe oryzae*: *Mo*Pl1 is required for full virulence of *Magnaporthe oryzae*

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The hemibiotrophic rice blast fungus *Magnaporthe oryzae* is the economically most important rice pathogen worldwide, severely affecting global food security. To combat the disease an in depth understanding of the infection mechanism of the fungus is mandatory.

During plant infection, the fungus utilizes different sets of secreted proteins, like e.g. cell wall degrading enzymes or effectors, which are thought to manipulate the plant immune response, all together enabling host colonization. Aiming at identification of novel virulence factors, we used in house data of proteome analyses of germination fluids from *M. oryzae* conidia and published data of genes solely found in plant-associated microorganism.
One of the candidate genes encodes a secreted pectate lyase of *M. oryzae*, termed MoPl1. The corresponding gene is expressed in the necrotrophic but not in the biotrophic stage of infection. Generation of mRFP-tagged mutants constitutively expressing *MoPL1* and loss of function mutants, respectively, revealed that *MoPL1* is required for full virulence of the pathogen. Furthermore, we analyzed *Mo*Pl1 by measuring the pectolytic activity, by using confocal laser scanning microscopy and by performing site-directed mutagenesis to trace its function in the plant-fungus interaction.

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

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Plant disease susceptibility can stem from a range of different factors. Pathogens employ effectors to suppress or bypass plant defenses, but plant proteins can also be involved in

facilitating infection. These proteins from the plant side, so-called susceptibility factors, are often essential for both plant development and full establishment of the pathogen. Barley RACB, a small monomeric G-protein, has been shown to be such a susceptibility factor. RACB has been extensively studied in the interaction with the powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*), which might target RACB directly to successfully infect plant cells. However, the molecular mechanisms behind RACB mediated susceptibility remain poorly understood. Using transgenic RACB overexpression plants and co-immunoprecipitation followed by MS-analysis, we identified new potential interaction partners and cellular pathways involved in RACB-mediated disease susceptibility. Among these, plant membrane-trafficking and phospholipid-binding proteins, as well as a fungal effector, were heavily overrepresented. Hence, we aim to identify the role of these proteins in the barley-*Bgh* interaction and provide insight into how phospholipids and membrane-trafficking can support fungal accommodation in barley epidermal cells.