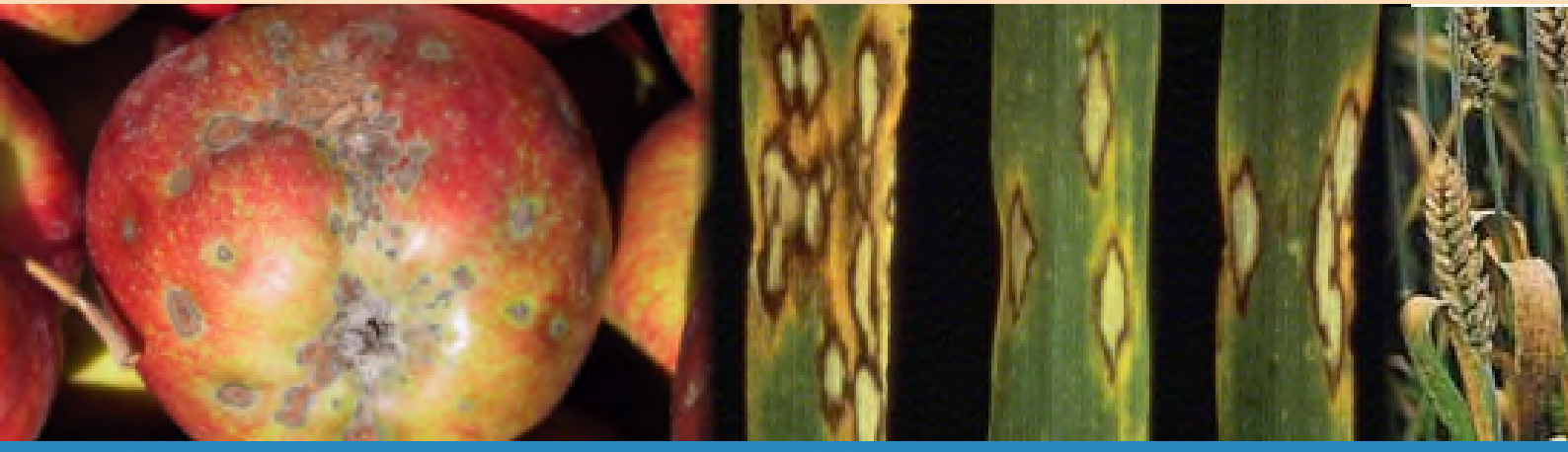
DPG | Spectrum Phytomedizin

Matthias Hahn / Anne-Katrin Mahlein (Hrsg.)

56. Jahrestagung des DPG-Arbeitskreises Mykologie und 40. Jahrestagung des DPG-Arbeitskreises Wirt-Parasit-Beziehungen 2021 – Online-Tagung



Zusammenfassungen der Arbeitskreisbeiträge

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Deutsche Phytomedizinische Gesellschaft e.V.

Matthias Hahn / Anne-Katrin Mahlein (Hrsg.)

56. Jahrestagung des DPG-Arbeitskreises Mykologie

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

2021

Zusammenfassungen der Arbeitskreisbeiträge

18./19. März 2021

Online-Tagung

PI (Persistent Identifier): urn:nbn:de:0294-jb-ak-2021-wpb-5

Arbeitskreise ‚Mykologie' und ‚Wirt-Parasit-Beziehungen' 2021

Die gemeinsame Tagung der Arbeitskreise ‚Mykologie' und ‚Wirt-Parasit-Beziehungen' fand am 18./19. März 2021 pandemiebedingt online statt.

Die nächste Tagung ist für den 10./11. März 2022 an der Technischen Universität München geplant.

Die Zusammenfassungen eines Teils der Beiträge werden - soweit von den Vortragenden eingereicht - im Folgenden wiedergegeben.

Leiter AK ‚Wirt-Parasit-Beziehungen': Matthias HAHN, Kaiserslautern
Leiterin AK ‚Mykologie': Anne-Katrin MAHLEIN, Göttingen

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

Tagung der beiden Arbeitskreise Wirt-Parasit-Beziehungen und Mykologie der Deutschen Phytomedizinischen Gesellschaft (DPG) als digitale-Videokonferenz, 18. und 19. März 2021

Nachdem wegen der Corona-Pandemie die Jahrestagung 2020 leider kurzfristig ausfallen musste, fand die Jahrestagung 2021 der Arbeitskreise »Wirt-Parasit-Beziehungen« und »Mykologie« der Deutschen Phytomedizinischen Gesellschaft e. V. am 21. und 22. März als digitale Videokonferenz statt. Diese wurde freundlicherweise von unserem Kollegen Prof. Ulrich Schaffrath (RWTH Aachen) und seinem Team technisch organisiert und ein reibungsloser Ablauf sichergestellt. Erfreulicherweise gab es mit 112 bzw. 60 Anmeldungen für die beiden Arbeitskreise und zeitweise über 145 zugeschalteten Teilnehmern einen außergewöhnlich guten Zuspruch zu dieser Tagung. Das Programm umfasste wie gewohnt vier Sektionen mit insgesamt 39 Beiträgen. Neben 30 Vorträgen gab es 9 sogenannte Poster-Pitches, bei denen die Autoren zunächst innerhalb von drei Minuten ihre Projekte vorstellten und später auf virtuelle Räume verteilt wurden, in die interessierte Teilnehmer des Treffens eintreten (und später auch wechseln) konnten, um das Poster bzw. die Kurzpräsentation zu diskutieren. Dieses digitale Format der Posterpräsentation wurde lebhaft angenommen und erlaubte einen direkten Austausch mit den Autoren. Im Rahmen des Treffens wurde Prof. Marco Thines (Senckenberg BiK-F und Goethe Universität Frankfurt) mit dem Wissenschaftspreis der DPG 2021 für seine Verdienste in der Erforschung filamentöser Schaderreger ausgezeichnet. Die Laudatio sprach Prof. Ralph Hückelhoven, 1. Vorsitzender der DPG. Anschließend hielt Herr Thines einen Gastvortrag über seine Arbeit, mit dem Fokus auf dem aktuellen Stand der Systematik der Oomyzeten, zu der er maßgebliche Beiträge geleistet hat. In einem weiteren Gastvortrag präsentierte Prof. Armin Djamei (Universität Bonn) seine Arbeiten über die Vielfalt und die Bedeutung der Effektorproteine des Maisbrand-Erregers *Ustilago maydis*.

Die in den Beiträgen vorgestellten Themen waren vielfältig und spannten einen weiten Bogen von der grundlagenorientierten Forschung, zu Diagnostikverfahren, über die Vorstellung von Schaderregern bis hin zu feldbasierter Forschung zu Auftreten, Epidemiologie und Erkennung von Krankheiten.

Das nächste gemeinsame Jahrestreffen der Arbeitskreise »Wirt-Parasit-Beziehungen« und »Mykologie« wird nach der zweifachen Verschiebung mit großer Wahrscheinlich am 10. und 11. März 2022 an der Technischen Universität München stattfinden; Gastgeber wird Professor Dr. Ralph Hückelhoven sein.

Prof. Dr. Anne-Katrin Mahlein
und Prof. Dr. Matthias Hahn

Liste der Vorträge

Sektion 1

1. Evolution and Diversity of plant pathogenic oomycetes - past challenges and future prospects
   Marco Thines (Senckenberg BiK-F und Univ. Frankfurt)
2. Nematode ascaroside ascr#18 primes plants for enhanced defense
   Andrea Mantai, M Manohar, F Schroeder, U Conrath, D Klessig (RWTH Aachen)
3. Comparative metabolomics of *Solanum lycopersicum* to elucidate anti-fungal defence mechanisms
   Lina Muñoz, C Meng, K Kleigrewe, R Hückelhoven, R Stam (TU München)
4. New insights into the *Verticillium* – plant interaction: the role of phytohormones and potential susceptibility factors
   Dirk Schenke, C Daguang (Univ Kiel)
5. Development of a molecular detection system for *Fusarium* spp., the causal agent of Fusarium root rot on soybean
   Daniela Hirschburger, C Trautmann, A El-Hasan, T Link, RT Voegele (Univ Hohenheim)
6. A Question of Time: Priority effects during co-inoculation of *Fusarium*, *Alternaria*, and *Pseudomonas* on wheat-ears
   Annika Hoffmann, M Koch, P Lentzsch, M E. H. Müller, C Büttner (HU Berlin)
7. Fusarium head blight (FHB) in wheat: effect of infection timing on disease development and mycotoxin accumulation
   Elias Alisaac, A Rathgeb, P Karlovsky, A-K Mahlein (Institut für Zuckerrübenforschung, Göttingen)

Sektion 2

8. Barley RIC proteins – scaffolds involved in RACB-mediated susceptibility to powdery mildew
   Stefan Engelhardt, M Kopischke, J Hofer, CP Igisch, K Probst, C McCollum, A Trutzenberg, R Hückelhoven (TU München)
9. Characterization of tissue specific LORE expression and LORE-dependent immunity in *Arabidopsis thaliana*
   Henriette Leicher, S Ranff (TU München)
10. Biochemical characterization of MLO2 in *Arabidopsis thaliana*
    Franz Leissing, N Huck, L Huang, R Panstruga, U Conrath, GFM Beckers (RWTH Aachen)
11. Mutations in Cyp51 of *Venturia inaequalis* and their effects on DMI sensitivity
    Mascha Hoffmeister, J Böhm, G Stammler (BASF)
12. Current studies on mechanisms causing lower demethylation-inhibitor (DMI) sensitivity of *Phakopsora pachyrhizi*
    Sarah Stilgenbauer, G Stammler, U Steiner (BASF)
13. Conidial task sharing – how two asexual spore types of the anthracnose fungus *Colletotrichum graminicola* shape development and pathogenicity
    Daniela Nordzieke, A Rudolph, C Schunke, A Sanken, G Beyer, S Pöggeler (Univ Göttingen)
14. *Os*JAC1 - Revealing the mode of action of a defense conferring rice protein
    Christian Kirsch, N Huwa, L Vogel, L Esch, B Sabelleck, T Classen, U Schaffrath (RWTH)
15. RGI-GOLVEN signaling promotes FLS2 abundance to regulate plant immunity
    Martin Stegmann, P Zecua-Ramirez, C Ludwig, H-S Lee, B Peterson, Z L. Nimchuk, Y Belkhadir, R Hückelhoven (TUM)

Sektion 3

16. Systematic effector studies and what we can learn from them
    Armin Djamei (INRES, Univ Bonn)
17. High nucleotide substitution rates associated with retrotransposon proliferation affect secretome evolution in smut pathogens
    Jasper Depotter, B Ökmen, MK Ebert, J Beckers, J Kruse, M Thines, G Doehlemann (Univ Köln)
18. The Sporisorium reilianum effector SAD1 causes an upregulation of abiotic stress and leads to loss of apical dominance by interfering with the function of a plant E3 ubiquitin ligase
    Nisha Agrawal, F Drechsler, T Reinicke, J Schirawski (Univ Jena)
19. Establishment of the first routine diagnostic assay for black root rot of strawberry caused by a species complex of fungi with *Cylindrocarpon*-like anamorphs
    Marco Loehrer, DS Petrescu, L Vogel, S Erwes, M Heupel, U Schaffrath (RWTH Aachen)
20. Automatic field scoring of Cercospora leaf spot using multispectral UAV image on time-series
    Abel Barreto, F Ispizua, S Paulus, M Varrelmann, A-K Mahlein (IfZ Göttingen)
21. Multiplex Real-Time PCR for detection of *Diaporthe*/*Phomopsis* Complex (DPC) species on soybean
    Bhenoush Hosseini, T Link, RT Vögele (Univ Hohenheim)

Sektion 4

22. Plant extracellular vesicles and their role in RNA-interference mediated plant protection
    Timo Schlemmer, A Koch (Univ Gießen)
23. Beyond RxLR effectors: Small RNAs and cysteine-rich proteins as novel weapons of the Arabidopsis downy mildew pathogen
    Florian Dunker, A Weiberg (LMU München)
24. The interplay of transposons, coding and noncoding RNAs governing the pathogenic life style of the barley powdery mildew fungus
    Stefan Kusch, J Qian, F Kümmel, M Erz, R Panstruga ((RWTH Aachen)
25. A new method for marker-free genome editing in *Magnaporthe oryzae*
    Alex Wegner, L Wirtz, T Leisen, M Hahn, U Schaffrath ((RWTH Aachen)
26. Defining the septin interactome during appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*
    Iris Eisermann, AJ Foster, P Derbyshire, F Menke, NJ Talbot (Saintsbury Lab, Norwich)
27. Epigenetic aspects of defense priming
    Sabine Engel, C Kirsch, U Conrath (RWTH Aachen)
28. Microbial antagonism in the *Arabidopsis th*aliana phyllosphere via Glycoside Hydrolase 25 (GH25) protein
    Priyamedha Sengupta, K Eitzen, E Kemen, G Doehlemann (Univ Köln)
29. Engineered Phylloplane Targeting of Antifungal Coumarins for Plant Protection
    David Spencer, P Schwinges, M Skrobanek, C Kipp, V Wanders, RT Biermann, S Dreischhoff, J Weber Böhlen, A Beesley, SF Beyer, H Schultheiss, U Conrath, CJG Langenbach (RWTH Aachen)
30. Tailored in planta biosynthesis of antifungal coumarins
    Jakob Weber Böhlen, P Schwinges, V Wanders, D Spencer, A Beesley, R Biermann, S Dreischhoff, Beyer SF, H Schultheiss, U Conrath, CJG Langenbach (RWTH Aachen)

Sektion Poster Pitch Session (3 min Kurzpräsentationen, gefolgt von break-out rooms für jedes Poster)

1. Christian Trautmann (Univ. Hohenheim): A UAV based monitoringsystem for plant diseases in field vegetable cultures
2. Stefan Thomas (Univ. Hohenheim): UAV based hyperspectral imaging combined with modern data analysis for non-invasive disease detection improves efficiency of precision farming
3. Ispizua Facundo (IfZ, Göttingen): Integration of optical, meteorological and environmental data to improve the detection of the occurrence of *Cercospora* - leaf spot disease
4. Anne-Katrin Mahlein (IfZ, Göttingen): FarmerSpace – ein digitales Experimentierfeld für den Pflanzenschutz
5. Martin Rieker (Univ. Hohenheim): Non-invasive, area-wide monitoring system for plant diseases and testing of new BCAs for NOcsPS cropping systems
6. Carolin Popp (Julius Kühn-Institut Dossenheim): Investigations of fungi as potential cause of sea buckthorn dieback in Northern Germany
7. Matthias Freh (RWTH Aachen): Exploring the molecular basis of the pleiotropic phenotypes associated with powdery mildew resistant barley and Arabidopsis *mlo* mutants
8. Louisa Wirtz (RWTH Aachen): Development of a method using CRISPR/Cas9 mediated gene disruption and simultaneous telomere vector-driven selection to detect essential genes in *Magnaporthe oryzae*
9. Thomas Leisen (TU Kaiserslautern): Functional characterization of the *Botrytis* secretome

Deutsche Phytomedizinische Gesellschaft e.V.

PROGRAMM

Donnerstag, 18.3.2021

13:00 Uhr **BEGRÜSSUNG, Laudatio für Prof. Marco Thines (Träger des Wissenschaftspreises der DPG)**

13:10 Uhr Marco Thines (Senckenberg BiK-F/ Univ. Frankfurt): Evolution and diversity of plant pathogenic oomycetes - past challenges and future prospects (Keynote lecture)

13:35 Uhr Andrea Mantai (RWTH Aachen): Nematode ascaroside ascr#18 primes plants for enhanced defense

13:50 Uhr Lina Marcela Munoz (TU München): Comparative metabolomics of *Solanum lycopersicum* to elucidate anti-fungal defence mechanisms

14:05 Uhr Dirk Schenke (Univ. Kiel): New insights into the *Verticillium* – plant interaction: the role of phytohormones and potential susceptibility factors

14:20 Uhr Daniela Hirschburger (Univ. Hohenheim): Development of a molecular detection system for *Fusarium* spp., the causal agent Fusarium root rot on soybean

14:35 Uhr Annika Hoffmann (ZALF; HU Berlin): A question of Time: Priority effects during co-inoculation of *Fusarium*, *Alternaria*, and *Pseudomonas* on wheat ears

14:50 Uhr Elias Alisaac (Univ. Bonn): *Fusarium* head blight (FHB) in wheat: effect of infection timing on disease development and mycotoxin accumulation

15:05 – 15.30 Uhr **Pause**

15:30 Uhr Stefan Engelhardt (TU München): Barley RIC proteins – scaffolds involved in RACB-mediated susceptibility to powdery mildew

15:45 Uhr Henriette Leicher (TU München): Characterization of tissue specific LORE expression and LORE-dependent immunity in *Arabidopsis thaliana*

16:00 Uhr Franz Leissing (RWTH Aachen): Biochemical characterization of MLO2 in *Arabidopsis thaliana*

16:15 Uhr Mascha Hoffmeister (BASF): Mutations in Cyp51 of *Venturia inaequalis* and their effects on DMI sensitivity

16:30 Uhr Sarah Stilgenbauer (BASF): Current studies on mechanisms causing lower demethylation-inhibitor (DMI) sensitivity of *Phakopsora pachyrhizi*

16:45 Uhr Daniela Nordzieke (Univ. Göttingen)**:** Conidial task sharing – how two asexual spore types of the anthracnose fungus *Colletotrichum graminicola* shape development and pathogenicity

17:00 Uhr Christian Kirsch (RWTH): OsJAC1 - Revealing the mode of action of a defense conferring rice protein

17:15 Uhr Martin Steegmann (TUM): RGI-GLV signalling controls FLS2 abundance to regulate plant immunity

Freitag, 19.3.21

8.30 Uhr Armin Djamei (INRES, Univ. Bonn): Systematic effector studies and what we can learn from them (Keynote lecture)

8:55 Uhr Jasper Depotter (Univ. Köln): High nucleotide substitution rates associated with retrotransposon proliferation affect secretome evolution in smut pathogens

9:10 Uhr Nisha Agrawal (Univ. Jena): The *Sporisorium reilianum* effector SAD1 causes an upregulation of abiotic stress and leads to loss of apical dominance by interfering with the function of a plant E3 ubiquitin ligase

9:25 Uhr Marco Loehrer (RWTH Aachen): Establishment of the first routine diagnostic assay for black root rot of strawberry caused by a species complex of fungi with Cylindrocarpon-like anamorphs

9:40 Uhr Abel Barreto (IfZ Göttingen): Automatic field scoring of Cercospora leaf spot using multispectral UAV image on time-series

9:55 Uhr Behnoush Hosseini (Univ. Hohenheim): Multiplex Real-Time PCR for detection of *Diaporthe*/*Phomopsis* Complex (DPC) species on soybe

10.10 - ca. 11.00 Uhr

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6: Carolin Popp (Julius Kühn-Institut Dossenheim): Investigations of fungi as potential cause of sea buckthorn dieback in Northern Germany

7: Matthias Freh (RWTH Aachen): Exploring the molecular basis of the pleiotropic phenotypes associated with powdery mildew resistant barley and Arabidopsis *mlo* mutants

8: Louisa Wirtz (RWTH Aachen): Development of a method using CRISPR/Cas9 mediated gene disruption and simultaneous telomere vector-driven selection to detect essential genes in *Magnaporthe oryzae*

9: Thomas Leisen (TU Kaiserslautern): Functional characterization of the *Botrytis* secretome

11:00 – 11.15 Uhr Pause

Freitag, 19.3.21

11:15 Uhr Timo Schlemmer (Univ. Gießen): Plant extracellular vesicles and their role in RNA-interference mediated plant protection

11:30 Uhr Florian Dunker (LMU München): Beyond RxLR effectors: Small RNAs and cysteine-rich proteins as novel weapons of the *Arabidopsis* downy mildew pathogen

11:45 Uhr Stefan Kusch (RWTH Aachen): The interplay of transposons, coding and noncoding RNAs governing the pathogenic life style of the barley powdery mildew fungus

12:00 Uhr Alex Wegener (RWTH Aachen): A new method for marker-free genome editing in *Magnaporthe oryzae*

12:15 Uhr Iris Eisermann (Saintsbury Lab, Norwich): Defining the septin interactome during appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

12:30 Uhr Sabine Engel (RWTH Aachen): Epigenetic aspcects of defense priming

12:45 Uhr Priyamedha Sengupta (Univ. Köln): Microbial antagonism in the *Arabidopsis thaliana* phyllosphere via Glycoside Hydrolase 25 (GH25) protein

13:00 Uhr David Spencer / Jakob Weber Böhlen (RWTH Aachen): Engineered phylloplane targeting of antifungal coumarins for plant protection / Tailored *in planta* biosynthesis of antifungal coumarins

13.20 Uhr Termin & Ort für Arbeitskreistreffen 2022, Verschiedenes

A) Abstracts der Vorträge

Evolution and Diversity of plant pathogenic oomycetes - past challenges and future prospects

Marco Thines

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Oomycetes are straminipilous organisms related to diatoms and seaweeds rather than opisthokont fungi. However, their filamentous members are devastating pathogens in agriculture. The organisms causing downy mildew of angiosperms are the most species-rich group of oomycetes and depend on living tissue for their nutrition. While it was widely thought that downy mildew species are specific only on the host family level, phylogenetic investigations and inoculation trials have generally confirmed specificity below the host genus level, with very few exceptions. This led to a reappraisal of early studies from about a century ago and the revision of several polyphyletic genera. A major challenge in investigating downy mildews is that, because of their obligate biotrophic nature, no large culture collections exist, rendering historic herbarium collections the primary source for investigations. Due to the high fragmentation of DNA in these samples, it is often difficult to obtain several loci for detailed phylogenetic investigations. But with whole genome sequencing based on short reads becoming ever more accessible, herbarium specimens now hold the promise for not only reconstructing the evolution of plant parasitic oomycetes, but also to trace their historic migration and epidemics in crops. Thereby, it will be feasible to disentangle the relationships in the paraphyletic genus *Phytophthora* and to learn from past pandemics, such as the one of *Phytophthora infestans* that triggered the Irish Famine in the 19th century. In addition, high throughput sequencing from environmental sequencing is prone to uncover or expand knowledge on neglected groups of soil-dwelling oomycetes, such as the genus *Lagena*.

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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Manosalva et al. (2015) Conserved nematode signaling molecules elicit plant defenses and pathogen resistance. Nature Communications, DOI: 10.1038/ncomms8795

Comparative metabolomics of *Solanum lycopersicum* to elucidate anti-fungal defence mechanisms.

Muñoz L, Meng C, Kleigrewe K, Hückelhoven R and Stam R.

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Tomato crops are attacked by diverse pathogens resulting in enormous losses of agricultural yield (Savary et al., 2012). Upon sensing the pathogens, plants mount various defence responses. In addition to well-studied responses such as a reactive oxygen species burst and strong up-regulation of Ethylene, Salycilic Acid, Jasmonic Acid, and other phytohormones, the secondary metabolites (SM) are expected to be involved in the quantitative resistance of plants to pathogens. Early leaf blight caused by species of genus Alternaria is a devastating disease in tomatoes and only very few cultivars show strong resistance against it (Chaerani & Voorrips, 2006). A large number of studies exist, investigating the role of SM in tomato. However, these focus predominantly on fruit and flavour-related compounds (Kim et al. 2014), and hence SM related to infection with Alternaria remain unknown. We applied metabolomics based on UPLC-QTOF mass spectrometry in combination with multivariate data analysis to

compare metabolic profiles of Solanum lycopersicum after treatments with A. alternata, A. solani and chitin (a general elicitor of anti-fungal plant defence responses) at 3 and 24 hours post inoculation (hpi). Here we present out latest analyses: we found that higher expression of features is observed after 24hpi. Most of the deferentially expressed features after treatment with A. solani are unique and the compounds shared between Chitin and A. alternata treatments may be related to defence response. We also observed a large overlaps between generic (chitin-triggered) anti-fungal defence responses, particular with those triggered by little virulent Alternaria alternata. When virulent A. solani was used for inoculation, more unique changes of the host metabolome were observed. We will discuss how these results might help resolve difference in quantitative resistance against closely related pathogens and might evidence pathogen-triggered manipulation of host SM responses.

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Chaerani R, Voorrips R. (2006). Tomato early blight (Alternaria solani): the pathogen, genetics, and breeding for resistance. *J Gen Plant Pathol* 72, 335–347. **https://doi.org/10.1007/s10327-006-0299-3**.

Kim, Dong Sub & Kwack, Yurina & Chun, Changhoo. (2014). Secondary Metabolite Profiling in Various Parts of Tomato Plants. *Korean Journal of Horticultural Science and Technology*. 32. 252-260. 10.7235/hort.2014.13165.

Development of a molecular detection system for *Fusarium* spp., the causal agent of Fusarium root rot on soybean

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In Germany, the production area of soybean has exponentially increased over the last decade. As a consequence, an increase in pathogen abundance is to be expected. Efficient soybean production requires better knowledge of associated pathogens that may result in considerable yield losses, both quantitatively and qualitatively. Although diseases may occur in different developmental stages, pathogens that cause a loss in seed quality and emergence are of special concern. One of the diseases that affect seed quality is Fusarium root rot (FRR). The causal agents of FRR on soybean are several *Fusarium* spp. The objective of this study is to develop a molecular detection method for *Fusarium* spp. on soybean using TaqMan like primer-probe technology.

DNA of ten isolates of seven different *Fusarium* spp. was extracted and the ITS1, Beta-Tubulin and TEF1 regions were amplified and sent for sequencing. Sequences were aligned and primer and probe pairs were designed using the software package BioEdit. In order to detect the ten different isolates, two primers and two probes were designed for Real-time PCR. The specificity was tested against other fungal pathogens like *Diaporthe* spp., *Rhizoctonia solani*, *Colletotrichum* spp. and *Sclerotinia sclerotiorum* and the primer efficiency was determined. Furthermore, the system has been succesfully adapted to seed samples.

Further experiments using the seed soaking method and duplex Real-time PCR are yet to be established.

A Question of Time: Priority effects during co-inoculation of *Fusarium*, *Alternaria,* and *Pseudomonas* on wheat-ears

Annika Hoffmann[1,2], Matthias Koch[3], Peter Lentzsch[1], Marina E. H. Müller[1], Carmen Büttner[3]

[1]Leibniz-Zentrum für Agrarlandschaftsforschung (ZALF) e. V. – Pilzliche Interaktionen; Eberswalder Str. 84, D-15374 Müncheberg

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Mycotoxigenic fungal pathogens *Fusarium* and *Alternaria* are a leading cause of loss in cereal production. This is countered by naturally occurring antagonists such as pseudomonads, which compete with fungi on the wheat-ear. However, studies investigating the interaction of these groups often neglect the temporal aspect of the infection process and the associated priority effects. In our study, which we would like to present, the focus was on the influence that the initial colonizer had on the subsequent ones (Hoffmann et al. 2021). *Pseudomonas* was emphasized in its ability to inhibit the growth of phytopathogenic fungi and their mycotoxin production. In a climate chamber experiment, wheat-ears were successively inoculated with two different strains (*Alternaria tenuissima*, *Fusarium graminearum*, or *Pseudomonas simiae*). Over three weeks, microbial abundances and mycotoxin concentrations were analyzed and visualized using Self Organizing Maps with Sammon Mapping (SOM-SM; Figure 1). This method, applied for the first time to microbial data, provided a simple way to visualize the multidimensional information in an easily understandable way. All three strains revealed different characteristics and strategies to deal with co-inoculation. Our findings on species-specific priority effects in a natural environment and the role of the mycotoxins involved contribute significantly to the development of effective biocontrol strategies.

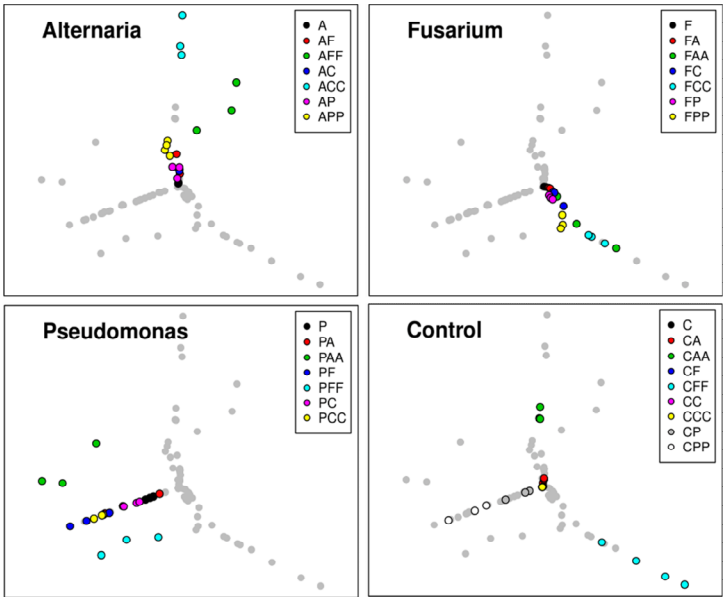

Figure 1 Abundance of co-inoculation experiment illustrated in SOM-SM. A dot indicates every single variant. C = control with 1/4 sterile Ringer's solution; A =*Alternaria tenuissima*, F = *Fusarium graminearum*, P = *Pseudomonas simiae*.

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Barley RIC proteins – scaffolds involved in RACB-mediated susceptibility to powdery mildew

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ROPs (Rho of Plants) are small monomeric G proteins that regulate a multitude of cellular processes in plants. The barley ROP RACB, which is required for full susceptibility of barley epidermal cells towards penetration by the biotrophic ascomycete *Blumeria graminis* f.sp. *hordei* (*Bgh*), has been shown to be involved in cell polarity and cytoskeleton reorganization (Engelhardt et al. 2020). 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 (Wu et al. 2001; Schultheiss et al. 2008). Here we describe yet uncharacterized barley leaf-expressed RIC proteins that can directly interact with RACB in yeast and *in planta*. This direct interaction is likely a prerequisite for RICs 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 distinct RIC proteins at the penetration site, specifically at the haustorial neck, during *Bgh* infection. Moreover, transiently overexpressed RIC proteins render barley epidermal cells more susceptible to *Bgh* in a RACB-dependent manner. Taken together, our data indicate that RIC proteins promote fungal infection into barley epidermal cells by potentially acting as scaffolds linking RACB to specific downstream signaling executors.

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Characterization of tissue specific *LORE* expression and LORE-dependent immunity in *Arabidopsis thaliana*

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Since most bacterial pathogens are not able to actively penetrate the plant epidermis, they must rely on natural openings for successful infection (Melotto *et al.*, 2008). To defend against pathogenic infection, plant cells are equipped with a two-layered immune system. Pattern-recognition receptors (PRRs) are part of the first level of this immune system. They perceive conserved molecular patterns, as for example microbe-associated molecular patterns (MAMPs) (Jones *et al.*, 2006; Zipfel, 2014). After binding of their respective elicitor, PRRs trigger a complex signalling cascade which results in a multitude of immune responses and confers basal resistance against various pathogens (Dodds *et al.*, 2010; Bigeard *et al.*, 2015). Enhanced expression of the PRR FLS2 in tissues targeted by bacterial entry indicated that potential entry points might be especially guarded to reduce bacterial invasion (Beck *et al.*, 2014). The PRR LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE) perceives bacterial medium-chain 3-hydroxy fatty acids with free 3-hydroxydecanoic acid being the strongest elicitor (Kutschera *et al.*, 2019). Previous studies showed that *lore* loss-of-function mutants were more susceptible to bacterial infection and that LORE might be particularly involved in pre-invasive immunity (Ranf *et al.*, 2015). To further characterize the role of LORE in plant immunity the natural *pLORE*-promoter was combined with two different reporter genes, *NLS-3xmVenus* and GUS_{Plus}. Investigation of the reporter lines provided new and surprising information about the natural *LORE* expression-pattern. Additionally, immune responses of plant lines with enhanced *LORE* expression in guard cells were analyzed to

further elucidate the function of LORE in pre-invasive immunity with special focus on stomatal immunity.

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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.

Mutations in Cyp51 of *Venturia inaequalis* and their effects on DMI sensitivity

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Venturia inaequalis causing apple scab, is the most important disease of apple worldwide in terms of cost and of control hassles. An important mode of action for control of *V. inaequalis* are the DMIs (sterol demethylation inhibitors). One mechanism leading to reduced DMI sensitivity of fungi is based on mutations in the target gene Cyp51. Up to now, Cyp51 mutations and their influence on the DMI sensitivity of apple scab has been investigated only sparsely.

To evaluate the potential variability of the Cyp51 gene of *V. inaequalis*, 122 monoconidial isolates were generated from field samples collected in apple orchards in different countries. The Cyp51 gene of all isolates was sequenced, and microtiter-tests were conducted to evaluate the sensitivity of the different isolates towards mefentrifluconazole, difenoconazole and myclobutanil. Furthermore, a new liquid culture method for *V. inaequalis* was established, which provides strong sporulating cultures as a basis for the sensitivity tests.

The sequence analysis revealed 15 different point mutations, each resulting in an amino acid change of Cyp51. Some of these are known as homologous mutations in other plant pathogens such as *Zymoseptoria tritici* or *Erysiphe necator*, where those mutations are involved in a reduced DMI sensitivity. Most of the mutations were in the YGYG-region, for example mutations Y443D or Y443C, but these had no or only low effects on DMI sensitivity. The mutations Y133F and M141T had more impact on DMI sensitivity with strongest effects on myclobutanil. Difenoconazole was less effected and mefentrifluconazole very low. The relevance of these mutations on the field efficacy is currently further investigated in greenhouse and field studies.

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Current studies on mechanisms causing lower demethylation-inhibitor (DMI) sensitivity of *Phakopsora pachyrhizi*

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The biotrophic basidiomycete *Phakopsora pachyrhizi* is the causal agent of Asian soybean rust (ASR), which has become a serious soybean disease in South America after its first appearance in 2001. Control of this disease is mainly based on fungicide applications, whereas demethylation inhibitors (DMIs), quinone outside-inhibitors (QoIs) and, in recent years, succinate dehydrogenase inhibitors (SDHIs) representing a large market. DMI fungicides have been the key component for ASR-control for many years. This ongoing selection pressure has led to an adaptation of *P. pachyrhizi* resulting in a continuous shift of the population towards reduced sensitivity to DMIs. In addition to overexpression of the *CYP51* gene, the most common resistance mechanism is the accumulation of point mutations in *CYP51*. In recent years, different haplotypes have been found, indicating that *CYP51* evolution is still ongoing. Mutations already known to lead to a lower efficacy and increased ED_{50}-values against DMIs are F120L, Y131F/H, K142R, I145F and I475T. Some of these mutations have already been found in combinations while others primarily occur alone (Schmitz et al., 2014). To classify the different haplotypes, for the first time single-spore isolates of *P. pachyrhizi* were obtained and investigated. The obtained isolates may contribute to further elucidation of the complex and not fully understood resistance mechanisms, especially of the *CYP51* gene. In addition to the fact that the fungus has two nuclei, it is also becoming increasingly apparent that several *CYP51* genes exist. The single-spore isolates will help to answer the question of how many *CYP51* alleles are present in *P. pachyrhizi* and provide detailed information on how the mutations in *CYP51* are linked. This information is of high importance for further resistance management.

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Conidial task sharing – how two asexual spore types of the anthracnose fungus *Colletotrichum graminicola* shape development and pathogenicity

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C. graminicola generates two distinct asexual spore types, oval and falcate shaped conidia, which can be generated specifically using spore-specific growth conditions in the lab. As our recent results show, oval and falcate conidia are adapted for efficient root and leaf infection, respectively. Basis for the organ-specific infection is a unique signal secretion pattern of both conidia, resulting in spore-specific development. Falcate conidia, generated on the surface of infected leaves, secrete mycosporine-glutamine. This natural compound prevents premature germination in the presence of numerous spore individuals before spreading, making the dissemination of the disease highly effective. Likewise, for oval conidia, the spore-specific secretion of a so far unknown germling fusion signal shapes development and infection: prior to leaf infection, oval conidia form germling networks by conidial anastomosis tubes (CATs). Since this process requires a high density of oval conidia at the infection point, these conidia are less efficient for leaf infection compared to falcate conidia. Additionally to the infection of above ground tissue, both conidia are infectious on roots. However, oval conidia are more efficient to cause disease symptoms like stunting when plants are grown in conidia-soaked substrate, mimicking the natural infection process. These results are supported by the analysis of chemotropic growth of conidia to maize root exudate using a 3D printed chemotropic device. In those analyses, oval conidia are highly efficient in detecting fungus-attracting signals present in maize root exudate, whereas falcate conidia show no active growth reaction to these compounds.

Taken together, evolution has equipped two distinct asexual spores of *C. graminicola* with the ability for efficient infection of distinct tissues of its host plant *Zea mays*. How the underlying communication processes between pathogen and host takes place, is part of our current investigations.

*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 broadspectrum 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 smaller than wild type plants as it 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, as e.g. split-YFP, are currently performed to verify the proposed protein-protein interaction.

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RGI-GOLVEN signaling promotes FLS2 abundance to regulate plant immunity

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Plant immune responses must be tightly controlled for proper allocation of resources for growth and development. In plants, endogenous signaling peptides along with low molecular weight phytohormones regulate developmental and growth-related processes. Recent research indicates that some of these peptides have regulatory functions in the control of plant immune responses. This classifies these peptides as phytocytokines as they show analogies with metazoan cytokines. Despite these principal findings, the mechanistic basis for phytocytokine-mediated regulation of plant immunity remains largely elusive. Here, we identify GOLVEN2 (GLV2) peptides as phytocytokines in *Arabidopsis thaliana*. By peptide application, GLV2-precursor overexpression and loss-of-function studies we show that GLV2 enhances sensitivity of plants to elicitation with the immunogenic bacterial flagellin epitope flg22. GLV2 is perceived by ROOT MERISTEM GROWTH FACTOR 1 INSENSITIVE (RGI) receptors and RGI3 forms an flg22-induced complex with the flg22 receptor FLAGELLIN SENSITIVE 2 (FLS2), suggesting that RGIs contribute to antibacterial immunity and are part of activated

pattern recognition receptor signaling platforms. GLV2 perception increases posttranscriptional FLS2 abundance and RGIs promote FLS2 protein accumulation. Thus, GLV-RGI signaling controls above ground plant immunity via a previously undescribed mechanism of phytocytokine activity.

Systematic effector studies and what we can learn from them

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Biotrophic plant pathogens evolve versatile strategies to subvert host immunity and to redirect metabolic flows in their favour. Plants co-evolve their immune system and together these interactions form complex dependency networks whose molecular dissection reveals often surprising connections. *Ustilago maydis* is a biotrophic maize pathogenic fungus inducing galls on all aerial parts of the plant. Its genome encodes for several hundred putative effector proteins, molecules that are send out by the pathogen to manipulate the host. In the past years my group studied systematically about 300 putative effectors of *U. maydis,* looking at their role in virulence, their interactions, their localisations and their activities in plants. Here I will report about some of the recent research highlights from this systematic study of an effectome and what we can conclude so far from them.

High nucleotide substitution rates associated with retrotransposon proliferation affect secretome evolution in smut pathogens

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Transposable elements (TEs) play a pivotal role in shaping diversity in eukaryotic genomes. The covered smut pathogen on barley, *Ustilago hordei*, encountered a recent genome expansion as its genome is over 30% larger than that of its close relative *Ustilago bromivora*. Using long reads, we assembled genomes of 6 *U. hordei* strains and found that the recent expansion can be mainly attributed to a single long terminal repeat retrotransposons (LTR-RT) family. TE proliferation is predominantly responsible for the large size of the mating type locus, which can be up to ~560 and ~470 kb in size for the *MAT-1* and *MAT-2*, respectively. The sequences of the mating type loci are largely distinct between the different mating types, as recombination is suppressed in these regions. To see if TEs also proliferated in closely related smut fungi other than *U. bromivora*, more smut species were de-novo sequenced. LTR-RT proliferation of these species occurred to a lower extent and halted a longer time ago compared to *U. hordei*. Intriguingly, high nucleotide substitution levels occurred more

clustered in the *U. hordei* compared to smut genomes with a more ancient LTR-RT proliferation. Moreover, differences in LTR-RT content between *U. hordei* and other smuts can mainly be observed in those genome regions where higher nucleotide substitution levels occurred. Remarkably, the high nucleotide substitution rate particularly affected the evolution of genes encoding secreted proteins as substitutions more frequently led to presence/absence polymorphisms and amino acid alterations.

The *Sporisorium reilianum* effector SAD1 causes an upregulation of abiotic stress and leads to loss of apical dominance by interfering with the function of a plant E3 ubiquitin ligase

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Sporisorium reilianum f. sp. *reilianum* is a biotrophic fungus that causes head smut disease in maize. After the infection of maize at the seedling stage, it spreads systemically inside the plant. Disease symptoms appear when the plants flower and include spore formation, phyllody, and an increase in the number of ears per plant due to the loss of apical dominance of maize ears. The effector protein SAD1 of *S. reilianum* is responsible for increasing the number of ears; however, its mechanism of action is unknown. The RNAseq analysis of young symptomless maize ears of 4 wpi plants infected with wild-type or Δ*sad1* strains of *S. reilianum* showed that SAD1 leads to upregulation of abiotic stress response genes of maize. Using the yeast secretion trap assay, we verified the functionality of the predicted secretion signal peptide of SAD1. The maize protein E3 ubiquitin ligase ZmRGLG1 was found as the potential interaction partner of SAD1 with the help of a yeast two-hybrid and a beta gal assay. AtRGLG1 and its homolog AtRGLG2 of *Arabidopsis. thaliana* are involved in the positive regulation of apical dominance as the *rglg1rglg2* double mutants of *A. thaliana* shows complete loss of apical dominance. AtRGLG1 and AtRGLG2 are also known to negatively regulate the abiotic stress response genes in *A. thaliana*. We confirmed the interaction of SAD1 with AtRGLG1, AtRGLG2, ZmRGLG1, and ZmRGLG2 by BiFC. In-vitro assays showed that SAD1 gets K63-polyubiquitinated by all four E3 ubiquitin ligases. K63 polyubiquitination was shown to have a role in the apical dominance. We generated transgenic *A. thaliana* lines expressing mCherry-SAD1 in the presence or absence of AtRGLG1 and AtRGLG2. Expression of *SAD1* in *rglg1* and *rglg2* single mutants led to more branches as compared to Col-0 expressing mCherry-SAD1. This suggests that SAD1 interferes with the function of RGLG1. We hypothesize that the interaction of SAD1 with RGLG1 and RGLG2 interferes with ubiquitination of the RGLG1 and RGLG2's target ERF53, a transcription factor that induces abiotic stress response genes. Lack of ubiquitination of ERF53 leads to prolonged-expression of abiotic stress response genes, which may lead to loss of apical dominance.

Establishment of the first routine diagnostic assay for black root rot of strawberry caused by a species complex of fungi with *Cylindrocarpon*-like anamorphs

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Black root rot of strawberry is caused by a species complex of fungi with *Cylindrocarpon*-like anamorphs and is a major constraint to strawberry production worldwide. Recently, *Ilyonectria* spp. and *Dactylonectria torresensis* were identified as species most probably causing the actual epidemic of black rot root in strawberries in Germany and The Netherlands. Infected plants show a retarded growth and smaller fruit sizes. The damaged root system might also be an entry port for other root pathogens from genera such as *Verticillium*, *Fusarium*, *Rhizoctonia Phytophthora*, and *Pythium*. So far, farmers rely on visual inspection of their planting material to separate infected plants form their nursery. Laboratory diagnostics are limited to tedious and time-consuming re-isolation and sequencing.

To resolve this issue, we developed the first PCR-based detection assay for strawberry black root rot in collaboration with a private company and the public plant protection service (Pflanzenschutzdienst NRW). Based on whole genome Markov Clustering (MCL) analysis of related species, gene sequences were selected which are common for species of the disease complex but absent from a wide range of other strawberry root-pathogenic fungi. We verified the specificity of the selected primer pairs and determined the detection limit. The new molecular diagnostic assay clearly discriminated infected plant material from healthy plants at early stages of infection. Currently it is developed into a routine diagnostic test with the aim to provide a tool for regular monitoring of the disease complex in plant breeding and propagation.

Automatic field scoring of Cercospora leaf spot using multispectral UAV image on time-series

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Visual rating of Cercospora leaf spot (CLS) is a time-consuming process that demands trained personal. Disease severity (DS) is one of the most reliable parameters 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 a multispectral camera system in time series. Flight mission was established to acquire images with a ground sampling distance (GSD) of 0.4 cm allowing the detection of single leaf spots on spectral images. In parallel, ground truth data (GT) on DS was assessed to validate an automatic scoring.

In this work, we present an image-processing approach that combining two machine learning classifiers based on partial least squares regression technique to differentiate vegetation and CLS affected tissue of the sugar beet canopy. As input features, vegetation indices, morphological features and image resolution allow high performance in time-series. The results show that it is possible to determine DS of affected sugar beet plots by using a machine learning approach. The comparison between GT data and UAV-based scoring presents a high non-linear correlation. The factor variety does not influence the performance. The approach has a tendency to underestimate the DS on average 19.34% less than the experts. This underestimation is probably related to differences in the scoring methodology, wherein scoring performed by experts, middle leaves are the object of assessment ignoring the presence or appearance of new leaves (Wolf and Verreet, 2002). Technical limitations of the equipment are especially the spatial resolution of the multispectral sensor (Jay et al. 2020). We conclude that multispectral UAV-systems are valuable tools to determine resistance of new sugar beet varieties on the field. This technology has the potential to reduce the so far very laborious work of visual scoring in the field and thereby possible accelerate the breeding process itself.

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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 is known as one of the most destructive soybean diseases, affecting seed quality and causing massive yield losses worldwide. The disease is caused primarily by *Diaporthe longicolla* along with other DPC species. Precise identification of the species of this complex is necessary for understanding the epidemiology of the disease and to develop better

control strategies. Based on the isolation of 32 DPC strains from DPC-damaged European soybean seeds we identified four species: *D*. *longicolla*, *D*. *caulivora*, *D*. *eres* and *D*. *novem*. These four species can be considered the principal DPC species on soybean in Central Europe. We now aim to develop a fast and accurate method to detect these pathogens via 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 and tested for specificity and efficiency using DNA from pure cultures of these species and other important soybean pathogens from genera *Sclerotinia, Colletotrichum, Fusarium*, *Uromyces*, and *Phakopsora*. Our primer-probe sets allow excellent discrimination of the different DPC species and can be used to detect and distinguish them in parallel using multiplex Real-Time PCR. The multiplex assay was tested on different plant material including healthy and infected soybean seeds or seed coats, soybean stems, and leaves. We now want to deveolop the assay into a standard procedure for testing soybean seeds and are planning comprehensive sampling to study the epidemiology of DPC species in Germany. Another aspect of our future research will be testing different soybean cultivars for their resistance responses against the different DPC species.

Plant extracellular vesicles and their role in RNA-interference mediated plant protection

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Protection by resistance mediating RNAs is an arising part of current research. Thereby, double stranded (ds)RNA resulting by endogenous transgene expression or by exogenous spray application leads to gene silencing in plant offending pathogens. The underlying mechanism of RNA interference (RNAi) is well understood; dsRNA is processed into 21-24 nucleotide (nt) long short interfering (si)RNAs by Dicer proteins. siRNAs are loaded into the RNA-induced silencing complex (RISC) resulting in mRNA degradation, translational inhibition, or DNA methylation of the target genes. Host induced gene silencing (HIGS) depends on integrated transgenes which produce double dsRNAs while spray induced gene silencing (SIGS) needs no production of gene modified organisms. Previously, we showed that the expression of a 791nt transgene (CYP3RNA) resulting in a dsRNA complementary to the three fungal CYP51 genes of *Fusarium graminearum* (Fg) and which are involved in the ergosterol biosynthesis led to resistance against Fg.

Here we focus on the inter-species transport of RNA by plant extracellular vesicles. Therefore, we isolated plant EVs from dsRNA expressing Arabidopsis plants which are resistant towards Fg and investigate the RNA cargo by RNA-seq experiments. We found transgene-derived siRNAs in the plant extracellular vesicles and the co-purified apoplastic fluid. Most of them

with a length of 21 or 22 nucleotids and preferentially starting with an uracil or adenin. Further studies of transgene expressing mutants of the endosomal sorting complex required for transport III (ESCRT-III) revealed a loss of transgene mediated resistance and no transgene-derived siRNAs after EV purification.

Beyond RxLR effectors: Small RNAs and cysteine-rich proteins as novel weapons of the Arabidopsis downy mildew pathogen

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Downy mildew diseases are common problems in the reliable cultivation of vegetables and fruits creating enormous economic loss and requiring high pesticide applications. Downy mildews are caused by obligate biotrophic oomycetes, that employ highly diverse and complex arsenals of effectors to overcome host immunity and reprogram host physiology. In contrast, oomycete effector research has almost exclusively focused on one class of cytoplasmic effectors that carry the RxLR amino acid translocation motif.

Here, we present the crucial importance of two poorly studied effectors classes: small RNAs and secreted cysteine-rich proteins. We revealed that small RNAs of *H. arabidopsidis* translocate into host cells, associate with the host Argonaute/RNA-induced silencing complex to suppress host immunity genes (Dunker et al., 2020). The small RNA exchange is likely bi-directional, as we successfully engineered plants to achieve host-induced gene silencing of *H. arabidopsidis* genes. We obtained a functional knock-down of the highly expressed, but uncharacterized effector *Cysteine-rich protein 1*. By further experiments we could uncover that this apoplastic effector most likely inhibits plant extracellular proteases and thereby prevents host programmed cell death (Dunker et al., 2021). These results underline the importance of small RNAs and non-RxLR effectors and provide a starting point for new targets of downy mildew disease control.

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The interplay of transposons, coding and noncoding RNAs governing the pathogenic life style of the barley powdery mildew fungus

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The obligate biotrophic ascomycete fungus *Blumeria graminis* causes the powdery mildew disease on grasses and cereals in a host-specific manner, with the barley powdery mildew (*B. graminis* f.sp. *hordei*, *Bgh*) only able to infect *Hordeum vulgare* (barley). Our near-complete genome assembly of *Bgh* revealed extensive copy number variation of candidate secreted effectors between *B. graminis formae speciales* and a recent lineage-specific expansion of transposable elements. These transposable elements make up >70% of the genome and can be the source of genetic variation and genome instability. Since the canonical fungal repeat-induced polymorphism (RIP) mechanism is absent in powdery mildews, *Bgh* may regulate transposable elements by epigenetic means or via noncoding RNA interference. Using high-throughput sequencing data we found evidence for small RNAs originating from transposons, suggesting that RNA interference contributes to transposon regulation. Importantly, we discovered long spliced antisense RNAs at loci of transposon replication genes. These may regulate transposons by RNA interference, may affect the chromatin status of transposons, or may serve as blueprints for novel genes or small peptides. We analyze the global co-expression profiles of protein-coding and long noncoding RNAs during the asexual lifecycle of the pathogen (germination to conidiation) and characterize their molecular function. Our work unravels how transposon regulation and gene invention contribute to virulence in fungal plant pathogens.

A new method for marker-free genome editing in *Magnaporthe oryzae*

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The ascomycete *Magnaporthe oryzae* also known as rice blast fungus is an economically important pathogen, severely affecting global food security. To unravel the mode of action of the infection process and to develop new methods to combat the disease, forward and reverse genetic approaches are essential. Unfortunately, experiments are often hindered by a limited

number of characterized selection markers. Here we report on a powerful method based on the co-transformation of a CRISPR/Cas9-ribonucleoprotein and a telomeric vector containing a resistance marker for the selection. In fungi, telomeric vectors are known to act like minichromosomes. The lack of centromeric elements results in an instability and loss of the vector as soon as the selection pressure is removed. This offers the opportunity to use telomeric vectors transiently and enables the possibility for the repetitive use of selection markers.

Due to this method, which provide a new and extremely efficient tool-box for the genome editing in the rice blast fungus, maker-free insertion of DNA at a specific locus (e.g. tagging of genes), site directed mutagenesis done directly in the fungus, integration of multiple genes or the deletion of multiple genes by marker-gene recycling and validation of essential genes will become possible.

Defining the septin interactome during appressorium-mediated plant infection by the rice blast fungus Magnaporthe oryzae

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The plant-pathogenic fungus *Magnaporthe oryzae* causes the devastating blast disease of rice, which leads to significant yield losses every year. It is a major threat to rice cultivation in more than 80 countries. The fungus forms a specialised pressure-generating infection cell called an appressorium, which penetrates the rice leaf using physical force to breach the tough leaf cuticle. Septins are GTP-binding proteins that play a major role in appressorium-mediated plant infection. Septins are components of the cytoskeleton that function by rigidifying the cortex of cells, scaffolding F-actin and localizing other proteins, including polarity determinants at the plasma membrane. In *M. oryzae*, four septins form a hetero-oligomeric ring structure at the base of the appressorium, which is essential for plant infection. In order to determine the role of septins in appressorium-mediated plant infection, we carried out a Hybrigenics based high throughput yeast two-hybrid assay and compared the results with those of *in vivo* immunoprecipitation mass spectrometry (IP-MS) experiments during appressorium morphogenesis. We aim to determine how *M. oryzae* septin complexes organise the appressorium pore to facilitate actin polymerisation, and act as lateral diffusion barriers to deploy polarity determinants at the point of infection. Comparative analysis of each data sets will allow us to define the septin interactome in unparalleled detail.

Epigenetic aspects of defense priming

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Defense priming refers to the enhanced capacity of cells to mobilize defense responses[1]. In plants and mammals, defense priming has been associated with epigenetic modifications in the promoter and promoter-proximal region of defense genes[2]. These modifications seem to provide an epigenetic memory, for example to previous infection, in that they prime the given gene for enhanced transcription[1,2]. For instance, upon localized bacterial infection, the trimethylation of lysine residue 4 in histone H3 (H3K4me3) increases in the promoter of defense gene *WRKY6* in systemic leaves. This is associated with strongly enhanced transcpription of *WRKY6* following infection of systemic leaves and with establishment of systemic acquired resistance (SAR).

To identify the histone methyltransferase(s) that write H3K4me3 during systemic defense priming, we focused on enzymes containing the conserved Su[var]3-9, Enhancer of zeste, and Trithorax (SET) domain. Such enzymes are known to methylate H3K residues[3]. We screened Arabidopsis knockout lines for attenuated defense priming and reduced SAR. Our findings provide further correlative evidence for the importance of H3K4me3 to defense priming and systemic immunity.

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Microbial antagonism in the *Arabidopsis thaliana* phyllosphere via Glycoside Hydrolase 25 (GH25) protein:

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Plant organs are colonized by a vast range of microbes, which interact among themselves to establish a community. Microbial interactions in turn can generate immune responses in plants against pathogens. Sampling of *A. thaliana* leaves (Agler et al., 2016) revealed an intricate network of bacteria, fungi and oomycetes, where an epiphytic yeast *Moesziomyces*

bullatus ex *Albugo* on *Arabidopsis* (*MbA)* was found to be a regulator of the phyllosphere community. *MbA* significantly reduced *A. thaliana* leaf infection caused by the oomycete white rust pathogen, *Albugo laibachii*. Transcriptome analysis identified a Glycoside hydrolase family 25 (GH25) gene activated in *MbA* when in contact with *A. laibachii in planta* (Eitzen et al., 2021). Further functional characterization of GH25 protein showed that the purified compound could reduce the *A. laibachii* infection in a manner similar to *MbA*. Our studies deepen the understanding of microbial interactions in the phyllosphere and carve a path for development of newer biocontrol strategies.

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Engineered Phylloplane Targeting of Antifungal Coumarins for Plant Protection

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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 phylloplane-borne 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 membrane transporter proteins resulted in rapid and efficient coumarin export in cell culture and whole plants. Transient co-expression of the identified *H. annuus* transporter with different coumarin biosynthetic genes in *Nicotiana benthamiana* revealed its capacity to not only provoke scopoletin export to the phylloplane, but also of structurally similar coumarin derivatives, hinting to a broad substrate range of the transporter. 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.

Tailored *in planta* biosynthesis of antifungal coumarins

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Coumarins and other phytoalexins are promising plant secondary metabolites for sustainable crop protection. In previous work we showed that the coumarin scopoletin effectively protects soybean from Asian soybean rust (SBR) and other diseases[1]. Here, we exploited sunflower methyltransferases (MTs) to synthesize isoscopoletin (an isomer of scopoletin) and scoparone (a scopoletin derivative) *in planta*. In *Nicotiana benthamiana* transient expression of MTs and a feruloyl-CoA 6'-hydroxylase (F6'H) lead to the accumulation of isoscopoletin or scoparone in transformed leaves. These two coumarins also protected soybean from SBR when coapplied. When exposed to light, isoscopoletin better withstood degradation than scoparone and scopoletin. Therefore, as scopoletin exerted the strongest antimicrobial activity against SBR and because isoscopoletin was most light stable, a combination of the two coumarins seems to be promising for effective plant protection. In addition, as scoparone in contrast to scopoletin and isoscopoletin is not inactivated by glycosylation we assume that a blend of the three here introduced coumarins provides a most potent approach for effective and eco-friendly crop protection.

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B) Abstracts der Poster

A UAV based monitoringsystem for plant diseases in field vegetable cultures

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Within the DiWenkLa project we aim to establish a dronebased monitoring system to detect disease causative plant pathogens in outdoor vegetable field cultures. The approach is to aquire multispectral images from the fields using a Micasens Dual camera sensor, attached to a DJI Matrice 210 V2 UAV.

Sunlight, which appears to the human eye as white light, consists of many different colours. These colours arise because of the different wavelength of their radiation. A healthy plant absorbs the photosynthetic active part of the sunlight, which are wavelengths between 380 till 780 nanometers. Only the part of the light appearing green, is reflected, which is the reason, why the colour of chlorophyll an therefore the plant itself appear green.

A plant which is infested by a pathogen or a pest is not able to use the full photosynthetic active radiation. This is the reason why the reflected light spectrum is different to the reflected light of a healthy plant. From laboratory trials it is known, that these changes are characteristic for the pathogens causing them, like a fingerprint.

What we do is to evaluate these pathogene characteristic changes for vegetable field cultures grown at the Filderebene near Stuttgart in Germany. The observated vegetable crops are cabbage and salad.

The aim is to establish a process that allows us to carry out an automated field monitoring system for detection of plant diseases. The obtained data then can be used to create infestation maps for a targeted fight against the on the field existing pathogens.

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

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

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 disease progression, and drone based multispectral measurements, which enable high throughput disease screening at 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 this study is to fly over entire fields with a 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.

Integration of optical, meteorological and environmental data to improve the detection of the occurrence of Cercospora - leaf spot disease

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Optical sensors and machine learning methods have shown high capabilities to detect Cercospora leaf spot (CLS) in sugar beet. However, weather and environmental conditions impact the disease development and spreading. Their possible contribution to improving the performance of detection is unknown. The spreading of *Cercospora beticola* occurs through conidiospores, which are mainly spread by wind and splashing water (Lawrence 1970). Conidia production is also affected in their incubation and release stage by two main parameters, the temperature and the relative humidity (Bleiholder und Weltzien 1971, 1972). The mentioned parameters must be taken into account to enhance CLS detection performance.

In 2020, a field experiment near Göttingen (Germany) was conducted to study the pathogen's spread and environmental interaction. Directly after sowing, plots were spot-inoculated with Cercospora beticola to emulate a natural infection. To quantify temperature and humidity, geo-referenced IoT-microclimate-sensors were installed to obtain information on the microclimate level. Furthermore, the quantification of spore production was determined by distributing spore traps in the field. Every week until the harvest, multispectral images (spectral bands: blue, green, red, red edge, and near-infrared) were taken with a camera mounted on an unmanned aerial vehicle (UAV). At the same time, as ground truth, visual scoring of the respective disease severity was carried out. During the preprocessing of the data, the optimized soil-adjusted vegetation index (OSAVI) was calculated. The heights of the plants were extracted from the digital elevation model. In addition, from the meteorological data growing degree days (Tb=1.1 °C) and the number of possible generations (Bleiholder und Weltzien 1971) were calculated. Furthermore, the accumulated concentration of spores was calculated from the data obtained from the spores. For the predictive regression modeling, a Random Forest method was used for training. Two models were created with different

datasets, first with UAV-only data and second with a combination of UAV data and environmental and spore sensors. Additionally, the most important variables of the models were determined.

In this work, the model for predicting disease severity of Cercospora from a workflow using only UAV imaging has shown positive results (Fig.1 A). However, the incorporation of environmental and spore-related information into the model showed a significant error reduction (RMSE reduction from 5.79 % to 4.94 %, Fig.1).

The factors that contributed most to the model were first the red and near-infrared band of the images and one vegetation index (OSAVI), followed by the height of the plant, information derived from temperature and humidity (degree days and number of possible generations (Bleiholder und Weltzien 1971), and the accumulative sum of the spore presence.

This work demonstrates the importance and usefulness of incorporate factors that influence plant-pathogen interaction to the CLS detection methods with optical sensors, thus achieving a robust model that can be generalized to other environments.

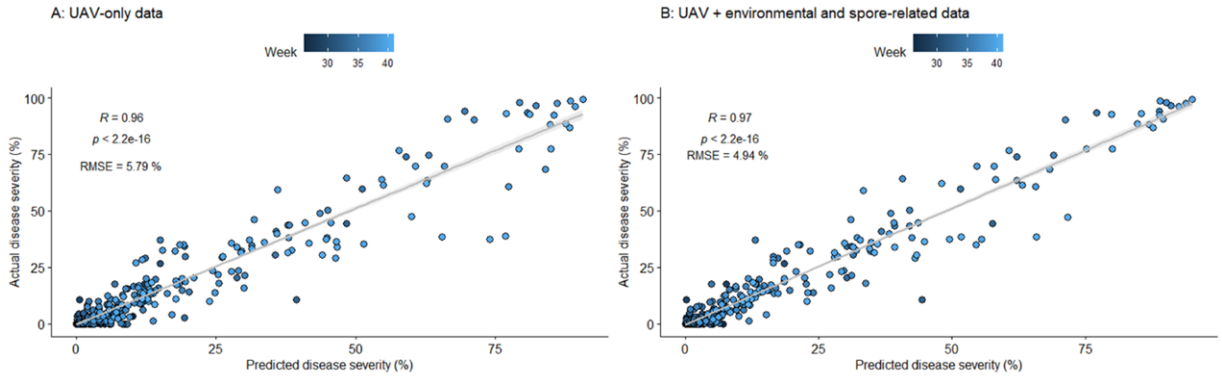

Figure 1: Scatter plot of predicted data versus field measured data for disease severity (%). Predictions from the model with only UAV data (A) and those from the combination of all data (B). Week= Weeks after sowing, R= Pearson correlation coefficient, RMSE= root-mean-square error

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Experimental field FarmerSpace – Assessment of digital plant protection from a farmer's perspective

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The project FarmerSpace is one of fourteen digital experimental fields, funded by the German Federal Ministry of Food and Agriculture to support digital technologies for crop production and animal husbandry and test their practicality. Within this scope, FarmerSpace uses and evaluates prototypes and market-available digital solutions for crop protection in practical applications in the sugar beet and wheat. With a focus on leaf diseases and weed management, the process from technical development to market entry of a new technology to widespread use on farms is accelerated. The usability of emerging technologies is scientifically evaluated, documented and published in a practice-oriented manner. The organizations involved in the joint project ensure both comprehensive evaluation and knowledge transfer.

An exemplary application of digital technologies in plant protection is phytopathometry, understood as the digital assessment of diseases and weeds. This involves the recording of image data using different camera systems on airborne or ground-based carrier platforms in the field and subsequent evaluation with machine learning methods and artificial intelligence - e.g. with neural networks in the case of very large training data volumes. This enables a high-resolution and position-accurate generation of field maps as well as a quantitative description of the occurrence of diseases and thus a targeted planning of a crop protection measure. These maps form the basis for future management measures, such as spot spraying or for a traceable visualization of crop development. One use case in farmerspace is the assessment and monitoring of foliar diseases of sugar beet such as Cercospora leaf spot with remote and proximal sensing. Unmanned aerial vehicles (UAVs) combined with state of the art multispectral and high resolution RGB-cameras enable a site specific detection of diseased spots. On the ground scale, mobile apps for disease identification are evaluated and compared regarding their accuracy and specificity. As additional information a IoT-sensor net has been established on the experimental field to assess environmental conditions on the micro-climate scale. As part of the field trial activities, systematic studies will be conducted in 2021 to evaluate the precision of existing disease detection methods and for implementing multi-sensor systems on a mobile platform. In a next step, a site specific spot-application should be specified based on sensor data information to control foliar pathogens efficiently. The project FarmerSpace is open for future collaboration with companies, start-ups and research partners.

Non-invasive, area-wide monitoring system for plant diseases and testing of new BCAs for NOcsPS cropping systems

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The use of chemical-synthetic plant protection products (csPPP) is seen increasingly critical by consumers and underlies increasing restrictions. However, their application is important to maintain high yield and good quality of crops, that are cultivated in cropping systems with close crop rotation and high intensity. Thus, the establishment of a new NOcsPS cropping system as alternative to conventional or organic farming systems is necessary. NOcsPS allows the targeted use of mineral fertilizer but renounces csPPP. One requirement for a sustainable realization of NOcsPS 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.

Pathogen specific changes in metabolism caused by *F. graminearum* in wheat and *S. sclerotiorum* in soybean will be detected by a hyperspectral camera setup under laboratory conditions. Hyperspectral data, which will be verified by molecular methods (qPCR), can be used for identification of pathogen specific spectral signatures. They provide information needed to develop a monitoring system that allows an early detection of plant pathogens under field conditions. Furthermore, several new BCAs will be characterized and tested for their efficacy to control the two pathogens, both in the greenhouse and in the field. These BCAs have the potential to replace csPPP in the long run.

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*.

Investigations of fungi as potential cause of sea buckthorn dieback in Northern Germany

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Sea buckthorn, *Hippophae rhamnoides*, is a diecious, hardwooded shrub, which is grown on an area of 723 ha in North-East Germany (Statistisches Bundesamt, 2021). The berries are characterized by high contents of vitamin c and antioxidants. Therefore, applications are pharmaceuticals, cosmetics and food products like juices or marmalades. Furthermore, wild

plants grown along the coastline of the Baltic Sea are of relevance for erosion protection and also form an important habitat and food source for various insects and animals. Since 2015, reports on the occurrence of sea buckthorn dieback, both in wild plants and plantations, have been accumulating, leading to serious ecological and economic consequences. Up to now, the cause of the plant death remains unexplained.

A joint project, HippRham, started in November 2020. It was established to investigate the cause of sea buckthorn dieback and to develop practical control strategies. The project partners are Landesforschungsanstalt für Landwirtschaft und Fischerei Mecklenburg-Vorpommern (LFA), Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern (LALLF), and JKI, Institute for Plant Protection in Fruit Crops and Viticulture. JKI focus will be on the fungal community potentially related to the dieback phenomena. In addition, phytoplasms and viruses will also be studied. For the fungal part, both a culture-dependent isolation approach and a culture-independent sequencing approach will be used. In a later stage, artificial inoculation experiments are planned, based on putative pathogens. So far, a total of 185 fungi were isolated from root and shoot material of different varieties and origin. Isolates were identified by ITS-PCR and Sanger-Sequencing and among others comprise *Clonostachys* sp., *Fusarium* sp., *Ilyonectria* sp., *Microdochium* sp., *Mucor* sp., *Penicillium* sp., and *Talaromyces* sp. (all from roots), and *Exophiala* sp., *Hymenopleella hippophaeicola*, *Neocucurbitaria* sp., *Penicillium* sp., *Phialemonium* sp., *Phoma* sp., and *Phomopsis* sp. (from shoots). For future studies, a method for DNA extraction from sea buckthorn plants was established.

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Exploring the molecular basis of the pleiotropic phenotypes associated with powdery mildew resistant barley and Arabidopsis *mlo* mutants

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Plant *mlo* mutants are known to show highly effective and durable resistance against powdery mildew pathogens. However, in some species such as barley and Arabidopsis, this type of resistance is accompanied by negative side-effects, so called pleiotropic phenotypes. These include developmentally controlled premature leaf senescence and necrosis, as well as the spontaneous formation of callose-containing cell wall deposits. While this has been known for more than 30 years, the molecular mechanism underlying this phenomenon has remained unknown (Wolter et al. 1993, Consonni et al. 2006). Our current working hypothesis includes the monitoring of MLO proteins as a so-called “guardee” by cytoplasmic immune sensors

(guard proteins). In case of the *mlo* mutant, the guard is proposed to be hyperactive due to the absence of its matching guardee. This project aims at elucidating the plant growth conditions that modulate the development of the pleiotropic phenotypes in barley and Arabidopsis. Apparently, *mlo* mutants of both species show enhanced pleiotropic phenotypes when exposed to abiotic stress like crowding and low temperature. Another aspect of this project is the search for the presumed guard proteins in both species.

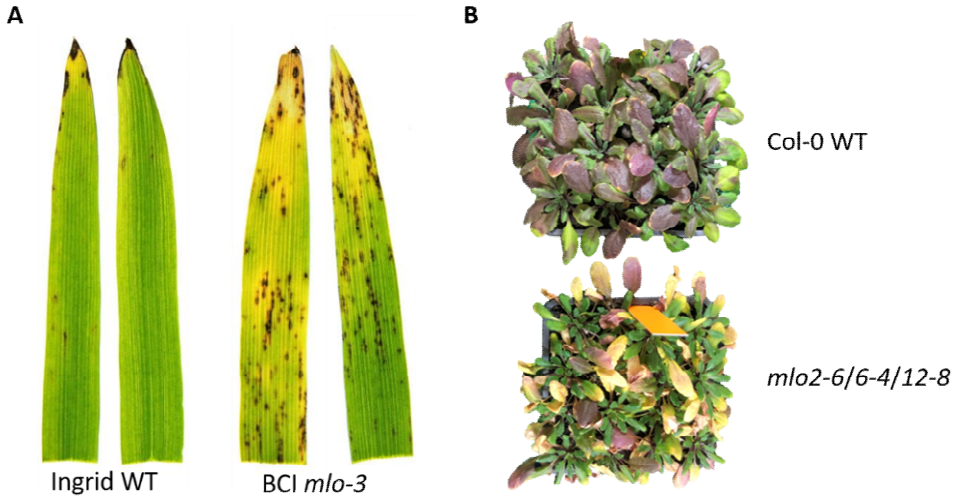

Figure 1: Leaf senescence and necrosis in barley and Arabidopsis *mlo* mutants. Comparison of **A)** primary leaves from 24-day old barley plants of cultivar Ingrid (wild-type) and the near isogenic back-crossed Ingrid (BCI) *mlo-3* line and **B)** 54-day old Arabidopsis plants of the genotypes Col-0 (WT) and *mlo2-6/6-4/12-8* triple mutant.

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Development of a method using CRISPR/Cas9 mediated gene disruption and simultaneous telomere vector-driven selection to detect essential genes in *Magnaporthe oryzae*

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Today, rice blast caused by the pathogen *Magnaporthe oryzae* constitutes the economically most significant threat to global rice production. The disease occurs irrespectively of location and conditions of rice farming and can lead to total crop losses which makes it to a serious problem to farmers and policymakers. This is particularly pressing in the context of climate

change, continued growth of global population and the stagnation or even decline in available agricultural land. Besides its economic relevance, *M*. *oryzae* became one of the model organisms for studying pathogen-plant interactions at the molecular level.

The study of genes that play an important role in fungal virulence is usually based on the generation of gene deletion mutants. An intrinsic problem for this type of reverse genetics is the analysis of essential genes which cause a lethal phenotype.

Methods to deal with essential genes are rarely described in general and in particular for *M. oryzae.* Therefore we aimed to develop a new method to analyze essential genes based on the co-transformation of a Cas9 ribonucleoprotein together with a telomere vector. In the cell such vectors replicate autonomously as centromere-free minichromosomes. The advantage of using telomere vectors which contain a pair of telomeres (pTEL) is their instability under conditions without selection pressure. Thus, after being transformed into fungal protoplasts the linearized telomere vector is maintained under selection conditions but got lost without selection. Essentially, this enables marker-free generation of transformants. Our strategy encompasses the presence of the gene of interest on a telomere vector expressed under the control of a strong promotor and the co-transformation concomitantly with a gene-deletion construct.

Thus, if the gene of interest is essential, the deletion of this gene will be compensated by the artificially provided gene on the telomere vector and even on not selective media only those transformants should survive which maintain the telomere vector.

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Functional characterization of the phytotoxic *Botrytis cinerea* secretome

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Botrytis cinerea is a plant pathogen characterized by necrotrophic infection of a large range of host plants. During penetration, it quickly kills the host cells and colonizes through the dead tissue. Several mechanisms have been demonstrated to be involved in host killing by *Botrytis* infection, including the release of phytotoxic metabolites and proteins, cell wall degrading enzymes, tissue acidification and the induction of defence responses culminating in the hypersensitive response, a plant-specific form of cell death. The precise role of the individual components during the infection is not yet fully understood. After the establishment of the CRISPR/Cas technology for genome editing in *Botrytis cinerea* (Leisen et al. 2020), we have

improved the method further by transforming double sgRNA-RNPs together with a transient selected telomere vector for repetitive marker-free gene deletions. This resulted in a highly efficient generation of homokaryotic, marker free single and double mutants within three weeks. We have constructed and characterized a series of up to ten-fold knockouts of genes encoding an array of necrosis inducing proteins. Surprisingly, preliminary data revealed only small reductions in virulence of the multi-k.o. mutants on different host tissues and in the phytotoxicity of their secretomes. This suggests that, in contrast to previous reports, these phytotoxins play only a minor role for necrotrophic infection, and that the killing of plant cells depends on so far unknown secretory compounds.

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