56th meeting of the DPG Working Group

"VIRUSKRANKHEITEN DER PFLANZEN"

March 11th and March 12th 2024

at

DLR Rheinpfalz, Neustadt a. d. Weinstraße

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DPG-working group leaders: Dr. Annette Niehl & Dr. Björn Krenz

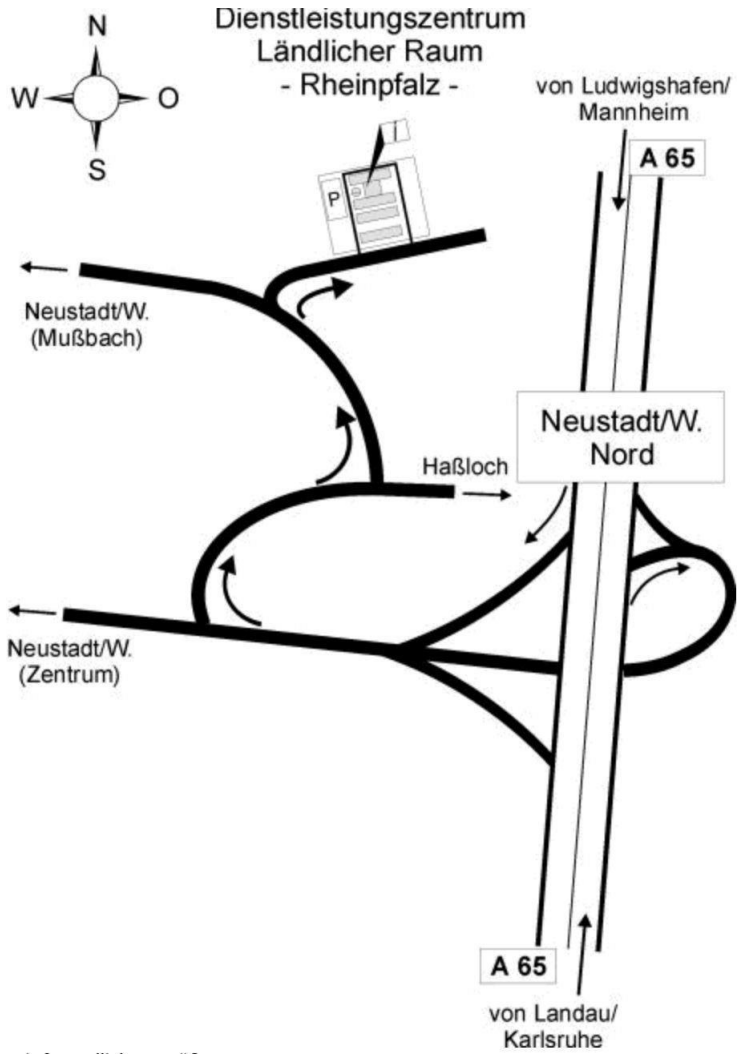
Organization & host: Prof. Dr. Gabi Krczal & team

RLP Agroscience

Dear colleagues,

We and the team of Prof. Dr. Gabi Krczal are welcoming you to the 56th meeting of the DPG Working Group " Viruskrankheiten der Pflanzen" in Neustadt a. d. Weinstraße on Monday, March 11th 2024 and Tuesday March 12th 2024.

The venue is the DLR Rheinpfalz:
Breitenweg 71, D-67435 Neustadt.
Aula



We look forward to a meeting that will benefit from numerous and diverse contributions, discussions and questions. The 2024 meeting will again be an excellent opportunity to network practice- and research-oriented plant virologists on scientific issues as well as applied problems in virus disease control. We all look forward to your continued support - especially from colleagues in the field of official extension!

We would like to point out to all active participants to keep exactly to the time and also to consider the discussions in terms of time.

Please note that the payment of 50 € to cover the catering (lunches & coffee/tea breaks) and rental costs **should be paid appropriately** at the meeting office, we have limited change. Thank you for your understanding.

We will be hosting the event through this link (Zoom):

https://us06web.zoom.us/j/82932186520?pwd=zoRuEuHkNqsJ1tX5cvaMmG9DblwjZS.1

or

Meeting-ID: 829 3218 6520; Kenncode: 749378

This link is reserved for conference participants.

With kind regards,

Annette Niehl & Björn Krenz

Our keynote speakers

Holger Puchta is director of the Joseph Gottlieb Kölreuter Institute for Plant Sciences at the Karlsruhe Institute of Technology (KIT) in Germany. After his study of biochemistry at the University of Tübingen and his PhD at the Max-Planck-Institute for Biochemistry in Munich he joined the laboratory of Barbara Hohn at the Friedrich Miescher Institute in Basel, Switzerland before he became in 1995 group leader at the Leibnitz Institute for Plant Genetics in Gatersleben (IPK), Germany. In 2000 he habilitated at the University Halle in Genetics. He was worldwide the first scientist to demonstrate that site-specific nucleases can be applied to induce controlled changes in plant genomes and one of leading scientists adopting the CRISPR/Cas technology to plants. Recently his group established CRISPR/Cas mediated plant chromosome engineering. He is since a row of years a "highly cited" researcher and was named "Pioneer of Plant Biotechnology" by the Plant Biotechnology Journal. He was awarded twice with a prestigious advanced grant of the European Research Council and most recently with the Reinhard Koselleck excellence funding of the German Research Foundation.

- Schmidt, Carla; Fransz, Paul; Rönspies, Michelle; Dreissig, Steven; Fuchs, Jörg; Heckmann, Stefan; Houben, Andreas; Puchta, Holger (2020): Changing local recombination patterns in Arabidopsis by CRISPR/Cas mediated chromosome engineering. In: Nature Communications 11, 4418.
- Beying, Natalja; Schmidt, Carla; Pacher, Michael; Houben, Andreas; Puchta, Holger (2020): CRISPR–Cas9-mediated induction of heritable chromosomal translocations in Arabidopsis. In: Nature Plants 6, 638-645.
- Rönspies, Michelle; Schmidt, Carla; Schindele, Patrick; Lieberman-Lazarovich, Michal; Houben, Andreas; Puchta, Holger (2022): Massive crossover suppression by CRISPR–Cas-mediated plant chromosome engineering. In Nature Plants 8,1153-1159.
- Schindele, Angelina; Gehrke, Fabienne; Schmidt, Carla; Röhrig, Sarah; Dorn, Annika; Puchta, Holger (2022): Using CRISPR-Kill for organ specific cell elimination by cleavage of tandem repeats. In Nature Communications. 13, 1502.

Klaus-Dieter Jany retired from his active job as the head (director) of the Centre of Molecular Biology at the Federal Research Centre for Nutrition and Food at Karlsruhe, now renamed Max-Rubner Institute at July 2008. The main research topic protein chemistry, enzymology, and applications of the genetic engineering in the agricultural and food area included safety assessment of genetically modified and novels food as well as the development of methods for detection genetically modified foods. 2008 entering into the active retirement. 2008-2018 voluntary work for the European Food Safety Authority (EFSA). Since 2008-2018 Vice President for Research and Teaching at Wadi International University, Syria. Since 2015 1st chairman of the association Wissenschaftskreis Genomik and Gentechnik e.V. (WGG). Frankfurt. The aim of the association is to present the complex topic of genome editing and genetic engineering in an understandable way and to inform the public, including politicians, about it objectively. The new genomic techniques and their legal regulation are currently the main topics.

- Jany K.D. and Höfer E. (2014): The German Beekeeper and Modern Plant Biotechnology. Verlag Dr Kovač, Hamburg; ISSN: 1435-6201, ISBN: 978-3-8300-8224-8

- Flachowsky G. und Jany K.-D. (2023): Gentechnik in der Ernährungswirtschaft – Beiträge zur globalen Ernährungssicherung und Bedeutung für die Wertschöpfungskette. Behr´s Verlag, Hamburg; ISBN 978-3-95468-8422 -5

Our keynote speakers

Frederik Gunnar Polzin ist Fachgebietsleiter des Fachgebietes Diagnose und Phytopathologie für gartenbauliche Kulturen in Rheinland-Pfalz am Dienstleistungszentrum ländlicher Raum Rheinpfalz**.** Das Fachgebiet übernimmt die hoheitlichen Diagnosen für den Erwerbsgartenbau sowie des öffentlichen Grüns. Weiter wird durch das Fachgebiet Erhebungen auf Quarantäneschadorganismen durchgeführt. Frederik Polzin studierte Gartenbaumanagement sowie Gartenbauwissenschaften an der Hochschule Geisenheim und promovierte anschließend im Bereich der molekularen Pflanzenwissenschaften an der Universität Göttingen. Danach absolvierte er das Agrarreferendariat in Rheinland-Pfalz und arbeitet seitdem als Fachgebietsleiter und Berufsschullehrer am DLR Rheinpfalz.

PROGRAMME 2024

Monday, 11. March 2024	
12:00 – 13:00	**Registration with snacks, soup and drinks**
13:00 – 13:10	**Welcome & conference information** *Annette Niehl, Gabi Krczal & Björn Krenz*
13:10 – 13:20	**Introduction of DPG & Junge DPG (please become a member)** *Annette Niehl & Björn Krenz*
	Section I: "Praxis & beyond" – chair: Renè van der Vlugt
13:20 – 13:45	**Keynote** **Rechtlich geregelte Viren - Übersicht und Einordnung** *Frederik Gunnar Polzin*
13:45 – 14:25	**Quarantäne und regulierte Nicht-Quarantäne Schaderreger: Aktuelle Herausforderungen in der Diagnose von Jordan-Virus und Grauburgundervirus** *Thierry Wetzel & Patrick Winterhagen*
14:25 – 14:40	**Laborvergleichsuntersuchung für Kartoffelviren: vom Probenaustausch einzelner Pflanzenschutzdienste zum etablierten internationalen Vergleich** *Wulf Menzel*
14:40 – 15:00	**Coffee & tea break – chair: Wulf Menzel**
15:00 – 15:15	**Befunde zu Viren in Sonderbeständen der Gemeinen Esche geben Aufschluss über das Virom von *Fraxinus excelsior*** *Marius Rehanek*
15:15 – 15:30	**TuYV-Infektion im Lichte der Vielfalt von Virusisolaten, Pflanzengenotypen und Blattlausklonen** *Torsten Will*
15:30 – 15:45	**Nanopore sequencing of viral RNA in its native form** *Paolo Margaria*
15:45 – 16:00	**Arabidopsis latent virus 1, a comovirus widely spread in Arabidopsis thaliana collections** *René van der Vlugt*
16:00 – 16:15	**Analyzing the diversity of the VPg-region of BaYMV isolates in Germany** *Claudia Strauch*
16:15 – 16:35	**Coffee & tea break – chair: Gabi Krczal und Carmen Büttner**
16:35 – 17:25	**Keynote** **Neue genomische Techniken – Regulierung und Anwendungen** *Klaus Dieter Jany*
17:25 – 17:45	**Investigating antiviral immunity in plant stem cells and germline** *Marco Incarbone*
17:45 – 18:00	**Investigations on a putative cysteine protease in the N-terminus of the celery latent virus (CeLV) polyprotein** *Svenja Lindenau*

18:00 – 19:00	**Poster session mit Aperitif** 1. No evidence for direct interaction between resistance protein Rz2 and its avirulence factor TGB1 from beet necrotic yellow vein virus in yeast-two hybrid *Kirstin Benjes* 2. A Luminex MagPlex xTAG assay to distinguish infectious from non-infectious viruses *Jan Bergervoet* 3. Ermittlung der Genomsequenzen neuer oder unvollständig charakterisierter Potyviren aus Zierpflanzen mittel Illumina HTS *Dennis Knierim* 4. Identifizierung eines neuen Allexivirus aus Ulme *Kira Köpke* 5. Highly curated and reliable E-probes for detection of hop pathogens in unassembled HTS datasets. *Ali Pasha* 6. Genetic diversity analyses of ash shoestring-associated virus (ASaV) in Germany *Sahar Nouri* 7. Risk of hop viroids in citrus-based plant-strengthening products *Swati Jagani* *8.* Genetic Diversity and Population Structure of the novel Aspen Mosaic-Associated Virus (AsMaV) in Populus Trees across Finland and Sweden *Shaheen Nourinejhad Zarghani* *9.* Mycoviruses suppress production of the mycotoxin deoxynivalenol by *Fusarium graminearum* *Simon Schiwek* *10.* Early transcriptomic response in sugar beet leaf tissue following aphid-mediated infection with beet yellows virus *Roxana Hossain* *11.* Integrated microscopy investigations aimed at elucidating interactions between viruses and their host within the plant nucleus *Christoph Sicking* 12. *Psammotettix alienus* as potential vector of pathogenic phytoplasmas *Britta Ruckwied* 13. Ornamentals matter: Impact of ornamentals for the One Health-concept and as virus reservoir in the Anthropocene *Katja Richert-Pöggeler und Sylvia Plaschil* 14. A risk assessment study of the virome of the tuberous crop Mashua (*Tropaeolum tuberosum*) *René A.A. van der Vlugt, Petra van Bekkum, and Annette Dullemans* 15. Studies on the N-terminal signal peptide of celery latent virus (CeLV) *Bianca Schulz*
20:00	**Conference dinner at Achat Hotel**

Tuesday, 12. March 2024	
08:25 – 08:30	**Welcome & conference information** *Annette Niehl & Björn Krenz*
	Section II: "resistance I" – chair: Christina Wege
08:30 – 08:45	**A step closer on the road to cross-protection Introducing mutations to find attenuated virus strains** *Mareike Rohde*
08:45 – 09:00	**Cassava Brown Streak Virus Resistance, Mechanisms and Potential Targets for Crop Improvement** *Samar Sheat*
09:00 – 09:50	**keynote (40 + 10 min)** **Applying CRISPR/Cas to Plants from Gene Editing to Genome Engineering** *Holger Puchta*
10min break	**Section III: "resistance II" – chair: Samar Sheat**
10:00 – 10:15	**E. coli expressed dsRNA of beet mosaic virus (BtMV) protects Beta vulgaris and Nicotiana benthamiana against the mechanically inoculated virus** *Dennis Rahenbrock*
10:15 – 10:30	**Exogenous application of dsRNA to control plant viruses and other pathogens and pests: it works! Now what?** *Khalid Amari*
10:30 – 10:45	**Recessive resistance against beet chlorosis virus is conferred by the eukaryotic translation initiation factor (iso)4E in Beta vulgaris** *Lukas Rollwage*
10:45 – 11:00	**Influence of inoculation time-point of BChV, BMYV and BYV and first results of BtMV mixed infection on sugar beet yield under field conditions** *Simon Borgolte*
11:00 – 11:30	**Coffee & tea break**
	Section VI: "-omics" – chair: Mark Varrelmann
11:30 – 11:45	**Insights into the molecular basis of beet curly top resistance in sugar beet through a transcriptomic approach at early stage of symptom development** *Omid Eini*
11:45 – 12:00	**Comparative transcriptome analysis of virus-resistant cassava lines infected with cassava brown streak virus (CBSV)** *Jessica Lilienthal*
12:00– 12:15	**Identification of the transcriptional landscape of the geminiviral infection in tomato leaves using single- cell RNA sequencing** *Huang Tan*
12:15 – 12:30	**Exploring the geminivirus proteome: composition, intra-viral connectivity, and functionality** *Shuyi Luo*
12:30 – 12:45	**Interplay between a polerovirus (BChV) and a closterovirus (BYV) infecting sugar beet** *Souheyla Khechmar*

12:45 – 13:05	**Relieving the burden of replication: selective autophagy protects host cells against virus-induced organelle remodeling** *Marion Clavel*
13:05 – 13:30	**Farewell & honors *by*** *Annette Niehl & Björn Krenz* **Coffee & tea and Snacks 2 go**

ABSTRACTS

Monday, 11. March 2024

Keynote

Rechtlich geregelte Viren - Übersicht und Einordnung

Polzin, F. G.

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Eingeschleppte Organismen gefährden die bestehende Landwirtschaft sowie die Biodiversität in den Gebieten, in welchen sie vorher nicht präsent waren. Aufgrund fehlender Anpassung der heimischen Pflanzen und Pflanzenbausysteme stellen diese invasiven Arten eine große Bedrohung dar und werden daher durch verschiedene Verordnungen und gesetzte innerhalb der Europäischen Union sowie der Nationalstaaten geregelt.

Hierbei spielen die Verordnungen der Europäischen Union (VO 2016/2031, 2019/2072, sowie 2019/1702 u.a.) eine besondere Rolle, da durch diese der Umgang mit Schaderregern für den gesamten Unionsraum geregelt wird.

Die Schadorganismen werden rechtlich in vier Gruppen unterteilt. Die Unionsquarantäneschädlinge, welche auch 20 prioritäre Schadorganismen enthalten, Schutzgebietsschädlinge, EU-Notmaßnahmen-Schadorganismen sowie geregelte Nicht-Quarantäneschädlinge (RNQPs).

Hinter jeder dieser Gruppen steht ein besonderes Regelwerk, welches bei Einfuhr, Monitoring und Auftreten beachtet werden muss. Dabei liegt der Hauptfokus auf den Unionsquarantäneschädlingen mit dem höchsten Schadpotential, direkt gefolgt durch die Schädlinge der EU-Notmaßnahmen und den RNQPs. Die Schutzgebietsschädlinge spielen in diesem Rahmen eine besondere Rolle, da diese im Unionsraum bereits stark verbreitet sind, jedoch in bestimmten Gebieten noch nicht vorkommen und diese Gebiete aus diesem Grund einen besonderen Schutzstatus genießen.

Quarantäne und Regulierte Nicht-Quarantäne Schaderreger: Aktuelle Herausforderungen in der Diagnose von Jordan-Virus und Grauburgundervirus

Wetzel T. und Winterhagen P.

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Als Quarantäneschaderreger gelten Organismen mit Schadwirkung auf Pflanzen in einem Gebiet, in dem sie bisher noch nicht auftreten oder noch nicht weit verbreitet sind. In der Regel handelt es sich hier um Organismen, die große Schäden in der Land- oder Forstwirtschaft verursachen und die biologische Vielfalt in den neuen Gebieten bedrohen können. Regulierte Nicht-Quarantäne Schaderreger (RNQP) hingegen kommen in Befallsgebieten vor und unterliegen einer gesetzlich geregelten phytosanitären Kontrolle, um eine weitere Ausbreitung zu verhindern und Schäden zu vermeiden. Erfahrungen und Probleme werden beispielhaft an zwei Viren vorgestellt: 1) Nachweis des Jordan-Virus (Tomato brown rugose fruit Virus, ToBRFV) nach EPPO Vorschriften im Rahmen der ISO 17025 Akkreditierung und mittels LAMP, sowie 2) die problematische Einstufung des Grauburgundervirus und dessen Diagnose. Das ToBRFV, das Tomaten und Paprika infiziert, steht seit 2020 auf der A2 Liste der EPPO. Da dieses Virus über Samen sowie mechanisch verbreitet werden kann, ist der schnelle und zuverlässige Nachweis des Virus wichtig um so früh wie möglich geeignete phytosanitäre Maßnahmen in betroffenen Beständen ergreifen zu können. Das Grauburgundervirus (GPGV) kommt in Weinreben vor und stellt aktuell ein ungeklärtes Risiko für den Weinbau dar. Das Auftreten der Symptomatik an Reben ist unzureichend geklärt, die Verbreitung durch natürliche Vektoren weitestgehend unbekannt und aktuelle Diagnosemethoden für Routinetestungen sind bezüglich Sensitivität bzw. Spezifität unzuverlässig, was eine Einstufung des Schaderregers erschwert.

Laborvergleichsuntersuchung für Kartoffelviren: vom Probenaustausch einzelner Pflanzenschutzdienste zum etablierten internationalen Vergleich

Menzel W.[1], Pastrik K.-H.[2], Steinbach P.[3]

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[2] *Landwirtschaftskammer Niedersachsen, Pflanzenschutzamt, Wunstorfer Landstraße 9, 30453 Hannover*
[3] *Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern, Pflanzenschutzdienst, Dorfplatz 1, 18276 Gülzow*

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Die praktische Verpflichtung der offiziellen Labore in der Pathogendiagnostik zur Akkreditierung nach ISO 17025 (Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien) hat zur Folge, dass diese ihre Kompetenz zum Nachweis bestimmter Erreger unter Anwendung der jeweils in ihren Laboren etablierten Nachweisverfahren durch eine regelmäßige Teilnahme an Laborvergleichsuntersuchungen (LVUs) unter Beweis stellen müssen. Derzeit gibt es in Europa keinen nach ISO 17043 (Anbieter von Eignungsprüfungen) akkreditierten Anbieter solcher LVUs für Pflanzenviren. Dies ist wahrscheinlich durch die große Diversität der benötigten Eignungsprüfungen einerseits, aber gleichzeitig quantitativ geringen Nachfrage andererseits begründet, und somit nicht kostendeckend umsetzbar. Aus der Diskrepanz, dass nach ISO 17025 akkreditierte Labore zur Teilnahme verpflichtet sind, es aber kein entsprechendes Angebot gibt, haben sich aus Testlaboren einzelner Bundesländer heraus in Eigeninitiative organisierte LVUs entwickelt, die auch von den Akkreditierungsstellen akzeptiert werden. Die erstmals in 2010 durchgeführte LVU für die sechs relevanten, als regulierte nicht Quarantäneerreger (RNQP) eingestuften Kartoffelviren PVY, PLRV, PVM, PVA, PVX und PVS begann mit einem Austausch infizierter Blattproben zwischen drei Laboren in Deutschland und hat sich zu einer jährlich organisierten, großen internationalen Eignungsprüfung entwickelt, für die in 2022 insgesamt 47 Probensätze an 31 teilnehmende Labore in 15 Ländern weltweit versendet wurden. In diesem Beitrag wird die Entwicklung dieser LVU für Kartoffelviren nachgezeichnet und die erzielten Ergebnisse werden diskutiert. Die einzelnen Labore nehmen mit den jeweils bei ihnen etablierten Protokollen teil, die von serologischen Verfahren (ELISA) über Schnelltests und Arrays bis zur RT-qPCR reichen. Dies liefert einerseits wichtige Informationen zur Kompetenz des teilnehmenden Labors, eröffnet andererseits aber auch Vergleichsmöglichkeiten innerhalb und zwischen Methoden. Im Laufe der Jahre erfolgte in vielen Laboren die Umstellung von der zeitaufwändigen Anzucht der Augenstecklinge mit anschließendem ELISA auf die zeitsparende RT-qPCR an der Kartoffelknolle. Hier half die LVU publizierte Verfahren für den Nachweis einzelner Viren als unzureichend zu identifizieren, da sie nicht alle in den LVUs eingesetzten Isolate eines Virus erfassten, so dass verbesserte Protokolle entwickelt und in weiteren LVUs erprobt werden konnten.

Befunde zu Viren in Sonderbeständen der Gemeinen Esche geben Aufschluss über das Virom von *Fraxinus excelsior*

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Pflanzenviren spielen in Waldökosystemen eine wichtige Rolle und können die natürliche Vitalität von Laubbäumen beeinträchtigen (Büttner et al. 2023). Als prädisponierender Faktor könnten sie auch am Eschentriebsterben (ETS) der Gemeinen Esche (*Fraxinus excelsior*) beteiligt sein, das als auslösender Faktor durch den pilzlichen Erreger *Hymenoscyphus fraxineus* verursacht wird. Da Eschenbestände europaweit im großen Umfang degenerieren, ist das große Verbundprojekt FraxForFuture initiiert worden, mit einem besonderen Fokus auf ETS infizierte Bestände (Langer et al. 2022). Innerhalb dessen wird in dem interdisziplinären FraxVir-Projekt insbesondere die Virusabundanz und -diversität in Eschensonderbeständen untersucht, um mögliche Auswirkungen von Virusinfektionen auf die Pathogenese des pilzlichen ETS-Erregers aufzuzeigen.
In drei aufeinander folgenden Jahren wurden Eschensonderbestände an fünf Standorten verschiedener Regionen Deutschlands untersucht, darunter Saatguterntebestände und Samenplantagen. Blattmaterial von Altbäumen, Jungeschen und Sämlingen wurde ebenso untersucht wie Saatgut, um Erkenntnisse über mögliche Übertragungswege der Viren zu gewinnen. Getestet wurde auf fünf Viren, nämlich die Nepoviren arabis mosaic virus (ArMV) und cherry leaf roll virus (CLRV), sowie neu entdeckte Viren in Eschen, wie das Emaravirus ash shoestring-associated virus (ASaV) (Gaskin et al. 2021; Rehanek et al. 2022), das Idaeovirus privet leaf blotch-associated virus (PrLBaV) (Navarro et al. 2017) und Viren der Cytorhabdovirus-Gruppe. Sequenzvergleiche von PCR-Amplifikaten, die von der taxonomisch relevanten Nukleokapsidregion generiert wurden, ergaben Unterschiede, die auf zwei verschiedene Cytorhabdovirus Spezies schließen lassen. Mittels Hochdurchsatz-Sequenzierung wurden darüber hinaus Signaturen bisher unbekannter Viren aus verschiedenen Familien identifiziert.
Die Ergebnisse des Virus-Screenings aus den ersten Projektjahren werden präsentiert.

LITERATUR

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TuYV-Infektion im Lichte der Vielfalt von Virusisolaten, Pflanzengenotypen und Blattlausklonen

Will T., Kern M., Breitkreuz C., Ziebell H., Thieme T.

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Pflanzenviren stellen in vielen Kulturen ein beträchtliches Risiko für den Anbau dar, da Infektionen i.d.R. zu signifikanten Ertragseinbußen führen. Das Turnip yellows virus (TuYV), welches in Deutschland großflächig verbreitet ist und hauptsächlich durch die Blattlausart *Myzus persicae* auf Raps übertragen wird, zeigt hierbei eine hohe Diversität zwischen verschiedenen Isolaten, was Untersuchungen für Groß Britannien und Australien zeigen konnten. Eigene Daten bestätigen eine genetische Diversität ebenfalls für in Deutschland gesammelte Isolate und zeigen zusätzlich anhand phänotypischer Daten, dass sich Isolate auch mit Hinblick auf ihre Virulenz, den relativen Virustiter aber auch Symptome wie z.B. Blattfärbung und Ertrag unterscheiden. Einzelne Virusisolate zeigen bereits in einigen resistenten Rapsgenotypen einen erhöhten Virustiter als auch phenotypische Effekte wie z.B. verringertes TKM und erhöhte Korngröße mit starker positiver Korrelation beider Merkmale im Falle einer Infektion. Eigene Versuche untermauern, dass Temperaturänderungen die in Sorten etablierte R54 Resistenz beeinflussen.

Nanopore sequencing of viral RNA in its native form

Margaria P.

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Since 2014, year of release of the first nanopore sequencer, the nanopore sequencing technology and applications in basic and applied research have undergone exponential growth. Advances for sequencing long nucleic acid molecules have led to substantial improvements in accuracy, read length and throughput over the last 10 years. A significant break-through, with profound impact in the field of virology, has been the ability of direct sequencing of RNA molecules. By avoiding the need of cDNA conversion or amplification in the library preparation, the approach paves the way to accurate analyses of haplotype composition of viral quasispecies, investigations on base modifications, transcript variants and RNA(s) architecture. The performance of the recently released Direct RNA sequencing kit will be presented on study cases in the context of end-to-end sequencing of virus genomes and resolving the complexity of viral populations within a single plant.

Arabidopsis latent virus 1, a comovirus widely spread in Arabidopsis thaliana collections

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Many plant biology studies involve Arabidopsis thaliana as a model plant. The Arabidopsis thaliana genome sequence became publicly available in 2000 (The Arabidopsis Genome Initiative, 2000). Since then, whole transcriptome sequencing has become one of the most common tools to decipher plant physiological processes. This untargeted approach can also reveal unexpected presence of other biological agents and provides information about possible unnoted infections and contaminations.
Particularly viruses can be hiding in plants and in seed material. The majority of well-studied viruses cause disease symptoms in agriculturally important crops with sometimes severe effects on plant morphology, physiology and yield. In nature however, plants are often infected with viruses that do not cause any apparent disease symptoms, so-called latent infections.
Here, we describe a comovirus; Arabidopsis latent virus 1 (ArLV1), that we encountered in A. thaliana RNA sequencing datasets generated in our labs and which was found to be widespread in other datasets obtained from sequence data repositories. We found that plants from several A. thaliana accessions, including the widely used accession Col-0 (CS60000), tested positive for ArLV1. We identified different isolates of the virus across the NCBI Sequence Read Archives and investigated disease symptoms, infectivity, plant growth and effects on the A. thaliana transcriptome and on abiotic stress resilience. Regular screening for the presence of this widely present – but unnoted – virus and further investigation of its possible effects is highly relevant for the plant science community working with A. thaliana.

Analyzing the diversity of the VPg-region of BaYMV isolates in Germany

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The yellowing disease in barley is caused by a complex of two viruses, barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV). Infection of barley fields with BaYMV can lead to yield losses of 50% in infested areas. The soil-borne vector Polymyxa graminis transmits BaYMV and forms resting spores containing infectious virus particles. To avoid crop losses due to BaYMV, resistant barley cultivars are used by farmers. Up to now, 22 resistance genes are described in barley and several of these are used in resistance breeding. However, BaYMV has efficiently overcome rym4 resistance in the past and currently, virus isolates, which can efficiently replicate in resistant plants with different backgrounds are being observed. An important factor, which can determine the resistance-breaking feature, is the viral VPg-region. We examine the VPg-region in the BaYMV genome by comparing virus-isolates from different fields and different barley cultivars. We expect to obtain insight into the viral diversity and response to resistance genes of these virus isolates. We hope to find changes in the virus isolates characteristic for resistance breaking and want to use this information to further monitor the presence and spread of these resistance breaking virus isolates in fields in Germany.

Neue genomische Techniken – Regulierung und Anwendungen

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Der Terminus „neue genomische Techniken“ (NGT) wurde weitgehend durch die EU-Kommission 2020 eingeführt und geprägt. Er löst den Begriff „neue Züchtungstechniken“ ab und zu den damals angewandten molekularbiologischen Methoden sind 2012 die Verfahren aus CRISPR/Cas und TALEN hinzugekommen. Mit diesen Verfahren können sequenzspezifisch Änderungen im Genom vorgenommen werden. Der EuGH hat die neuen genomischen Techniken als gentechnische Verfahren eingeordnet. Anwendungen der Gentechnik sind seit 1990/2001 durch die Freisetzungsrichtline streng geregelt. Diese Regelung beruht auf dem damaligen Stand von Wissen und Technik. Die Richtlinie wurde nie an neue Entwicklungen angepasst. Die EU-Kommission hat erkannt, dass das bestehende Gentechnikgesetz den NGT nicht mehr gerecht wird. Daher möchte sie für Anwendungen der NGT ein spezielles Gesetz (lex specialis) auf den Weg bringen. Das Gesetzgebungsverfahren in der EU ist komplex und neben der Kommission sind das EU-Parlament sowie die EU-Mitgliedstaaten dabei eingebunden. Am 05.07.2023 hat die Kommission einen Vorschlag für die Regulierung gewisser neuer genomischer Techniken vorgelegt. Der Vorschlag sieht die Einführung von zwei Kategorien von genomeditierten Pflanzen vor und beiden Kategorien ist gemeinsam, dass die modifizierten Pflanzen keine „artfremde“ genetische Information enthalten. Kategorie 1., die NGT-1-Pflanzen, sollen nach Überprüfung Pflanzen aus konventioneller Züchtung gleichgestellt werden. Sie sollen nicht mehr den Regelungen aus den einschlägigen Gentechnikgesetzen unterliegen. Sie unterliegen somit keiner speziellen Kennzeichnung und Rückverfolgbarkeit. Lediglich Saatgut soll zum Schutz des ökologischen Landbaus gekennzeichnet werden. Eine Art der Sicherheitsprüfung von NGT-1-Pflanzen wird in Registrierungsverfahren vorgesehen und eine Transparenz für alle Marktbeteiligten soll durch ein öffentliches Register und eine Identifizierungsnummer erreicht werden.

Kategorie 2., die NGT-2-Pflanzen, sollen ähnlich wie gentechnisch veränderte Pflanzen reguliert werden.

NGT-Pflanzen dürfen nicht im ökologischen Landbau verwendet werden. Generell dürfen die Mitgliedsstaaten den Anbau/Vermarktung von zugelassenen NTG-Pflanzen nicht behindern bzw. verbieten.

Am 07.02.2024 hat das EU-Parlament den Kommissionsvorschlag mit Änderungen keine Patentierung von NGT-Pflanzen, eindeutige Kennzeichnung, Rückverfolgbarkeit) angenommen. Nun liegt der weitere Gang des Verfahrens in der Verantwortung der Mitgliedsstaaten, die bislang sich noch nicht auf einen gemeinsamen Standpunkt zu dem Vorschlag einigen konnten.

In einer Reihe außereuropäischen Staaten werden genomeditierter Pflanzen, sofern sie keine „artfremde“ genetische Information enthalten, nicht als nicht gentechnisch verändert eingestuft und sie unterliegen einschlägigen pflanzenrechtlichen Regulierungen.

Die neuen genomischen Techniken werden in vielen Ländern in der Forschung und Anwendungen in der Pflanzenzüchtung genutzt. Im September 2023 listet die Datenbank von EU-SAGE (https://www.eu-sage.eu/genome-search) 787 publizierte Studien mit landwirtschaftlich relevanten Anwendungen in 70 unterschiedlichen Kulturpflanzenarten auf. Die Bandbreite dieser Anwendungen umfasst praktisch alle denkbaren Zuchtziele, von Krankheitsresistenzen über Anpassung an klimatischen Stress bis hin zu veränderten Inhaltsstoffen der pflanzlichen Produkte.

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Investigating antiviral immunity in plant stem cells and germline

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Stem cells are essential for the development and organ regeneration of multicellular organisms, so their infection by pathogenic viruses must be prevented. Accordingly, mammalian stem cells are highly resistant to viral infection due to dedicated antiviral pathways including RNA interference (RNAi). In plants, a small group of stem cells harbored within the shoot apical meristem (SAM) generates all postembryonic above-ground tissues, including the germline cells. Many viruses do not proliferate in these cells, yet the molecular bases of this exclusion remain only partially understood. In our recent main body of work, we show that a plant-encoded RNA-dependent RNA polymerase, after activation by the plant hormone salicylic acid, amplifies antiviral RNAi in infected tissues. This provides stem cells with RNA-based virus sequence information, which prevents virus proliferation. Furthermore, we find RNAi to be necessary for stem cell exclusion of several unrelated RNA viruses, despite their ability to efficiently suppress RNAi in the rest of the plant. In parallel lines of research we developed cutting-edge live imaging techniques to track virus movement through meristems and flowers in three dimensions and over time, which allow unprecedented insight into the dynamics of virus infection. Finally, we developed biological tools to investigate the factors protecting plant germlines from infection, which block the vertical transmission of infection by many viruses through plant generations yet remain largely unknown.

Investigations on a putative cysteine protease in the N-terminus of the celery latent virus (CeLV) polyprotein

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CeLV is the only species in the genus Celavirus (Potyviridae) that shows few sequence similarities to other Potyviridae members. Potyviridae members encode one or two proteases in the N-terminus (e.G. P1 & HC-Pro) and one main protease (NIa-Pro) in the C-terminal region that cleave the polyprotein into functional units. The amino acid sequence of the postulated 2nd protein in the N-terminus of CeLV suggests that it is a cysteine-type protease. To investigate the protease activity, potentially catalytically active amino acids (C627, H698 or H700) and the predicted cleavage site (AIVG/G) are mutated and cleavage activity was tested in vitro. For this, cell-free in vitro protein expression with subsequent visualization by SDS-PAGE was used. To investigate the effects of the introduced mutations on CeLV infection in plants, all mutations were cloned analogously into a full-length clone and tested in plants using Agrobacterium- mediated transformation with subsequent RT-PCR detection. It is hypothesized that when cleavage activity is suppressed, no systemic infection could be detected. In vitro, it was shown that in addition to a cysteine (C627), a histidine (H700) is also mainly responsible for the cleavage activity and the predicted cleavage site (AIVG/G) is correct. However, the results of the infection experiments in plants indicate that a second, closely localized histidine (H698) may also be used, as the two single mutations have shown no effect on infectivity in N. benthamiana and could be verified unaltered by sequencing. Folding studies in Alphafold support this hypothesis and show similar protein folding to the HC-Pro protein of PPV and TuMV.

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A step closer on the road to cross-protection
Introducing mutations to find attenuated virus strains

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Today, vegetable crops such as tomatoes, are often produced in greenhouses in highly specialized and densely planted systems. While this increases production efficiency, it also makes these crops especially vulnerable to emerging viral diseases. The tomato brown rugose fruit virus (ToBRFV) is a tobamovirus that has spread quickly since its first documented occurrence in 2014 (Luria et al. 2017) and has the potential to cause severe losses in tomato production. It is not recognized by the Tm-2^2 proteins, which prevent infection of tomato with other tobamoviruses, such as tobacco mosaic virus (Luria et al. 2017). Similar to other viruses of this family, ToBRFV it is easily transmitted mechanically and can be spread quickly by cultivation methods, such as pruning (Salem et al. 2022). Multiple strategies to deal with such challenges and manage viral diseases are investigated in the VIRTIGATION project. Cross-protection describes a special case of viral interaction where an initial infection with a mild isolate can prevent the establishment of a more severe isolate or related virus in the same plant.
We explore the development of ToBRFV cross-protection strains, which cause no or mild symptoms in host plants, from the severe wild-type isolate using nitrous acid as a mutagenic agent. Currently we are evaluating isolates with differing disease phenotypes to their parent strain. In the next step, their use as a protective agent against ToBRFV, as well as their long term stability will be tested. The application of nitrous acid is classified as a non-GMO method and has the potential of generating mild strains that can be used as plant protection agents in Europe and elsewhere, complementing hygiene measures and breeding efforts. With a varied toolkit of tested strategies, a fast response to emerging threats to food production is possible.

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Cassava Brown Streak Virus Resistance, Mechanisms and Potential Targets for Crop Improvement

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Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) are causative agents of cassava brown streak disease, leading to root necrosis and the destruction of edible tubers. We have identified resistance against these pathogens in South American germplasm lines, targeting either one or both virus species. In resistant plants, virus replication is hindered, with confinement to phloem companion cells and no translocation to neighboring parenchyma tissues. Our study aims to characterize resistance mechanisms against both viruses in cassava. Using cDNA clones of infectious viruses and modified virus genomes we tracked infection processes in cassava and *N. benthamiana*, to unravel viral and host genes associated with resistance responses. Notably, we highlight the Ham-1 gene's pivotal role in virus replication, movement, symptom expression and resistant in cassava. To understand plant-side resistance mechanisms, various experiments was conducted, including Genome-Wide Association Studies (GWAS), mapping by sequencing and small RNA analysis on resistant and susceptible plants. Weak signals found in GWAS and GBS on chromosome 11 implicated NLRR genes as potential players in CBSV resistances. However further signals were observed on chromosome 3 associated with UCBSV which warrants further verification. Using small RNA sequencing, we identified novel miRNAs which implicates lipid transfer genes with potential roles in plant immunity. Our findings contribute to a deeper understanding of both viral and host factors governing resistance against CBSV and UCBSV

Using CRISPR/Cas for plant breeding: From gene editing to chromosome engineering

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Till today CRISPR/Cas nucleases have been applied to plants mainly to mutate genes for the improvement of traits. As these enzymes are originating from human photogenic bacteria, their activity optimum is far above plant cultivation temperatures. By mutagenesis we were able to optimize Cas12a enzymes for plants use, resulting in much higher frequencies of mutation induction but also gene targeting by homologous recombination. Besides improving single traits, breeding also requires the breaking or establishing genetic linkages on the chromosome level. Using CRISPR/Cas, we were able to change genetic linkages by inducing heritable translocations in the Mb range between heterologous chromosomes in *Arabidopsis thaliana*. Recent improvements in sequence analysis of crop plants reveal that multi Mb long inversions occur with high frequency between different genotypes, leading to crossover suppression. We were not only able to demonstrate that inversions up to almost chromosome size can be achieved in *Arabidopsis*, but also meiotic recombination can be redirected this way. Thus, on one side a recombination dead region could be reactivated after 5000 years and on the other almost a complete chromosome could be excluded from genetic exchange. In the future, CRISPR/Cas-mediated chromosomal engineering will allow us to restructure plant genomes according to our needs for breeding. . Finally, we developed a new technology based on DSB-induced genome elimination for tissue engineering named CRISPR-Kill, allowing us to induce targeted cell death in different organs at selected developmental stages. Recently we were able to set up an inducible CRISPR-Kill system, allowing also the temporal control of cell death.

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E. coli expressed dsRNA of beet mosaic virus (BtMV) protects *Beta vulgaris* and *Nicotiana benthamiana* against the mechanically inoculated virus

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The spread of virus yellows (VY) and beet mosaic virus (BtMV, *Potyvirus*) in German sugar beet growing areas by the main vector *Myzus persicae*, and recent policy developments to reduce the use of chemical pesticides, highlight the importance of exploring alternative control strategies. One possible solution is to use viral double-stranded RNA (dsRNA) molecules to induce RNA interference (RNAi), a natural defense mechanism used by plants, to protect themselves against virus infection. By spraying plants with small amounts of viral dsRNA, we can initiate this natural defense mechanism and induce the plant's antiviral RNAi machinery. Exogenous application of dsRNA molecules has been shown to be effective against a wide range of viruses in several plant species, including potyviruses (Worral et al. 2019). However, dsRNA sprays have not yet been tested in *Beta vulgaris* or against beet infecting viruses.

In this study, we describe the production of two different dsRNA molecules in *E. coli* HT115 (Timmons et al. 2001), homologous to the partial nuclear inclusion body B (NIb) gene and the proteinase P1 gene of BtMV, based on its small RNA-profile. The dsRNA can be used as an RNA spray to inhibit the replication and spread of BtMV in *B. vulgaris* and *N. benthamiana*. Promising applications, where BtMV was mechanically inoculated on the same leaf 24 hours after the dsRNA spray, showed a general effect of the spray on BtMV susceptibility in both tested plant species. Further experiments demonstrated that even small amounts of dsRNA (6 µg) result in a significant reduction of the infection rate in *N. benthamiana*. However, the effect on systemically applied BtMV only causes a delay in virus infection.

Our results suggest that the use of dsRNA sprays may be a promising strategy for controlling viruses that affect beets, with a particular focus on the P1 region identified via small RNA sequencing and mapping. Moreover, we have the capability to detect specific virus derived small interfering (vsiRNAs) originating from the applied dsRNA and to observe its systemic movement in the plants. Further studies will test alternative dsRNA molecules and formulations to enhance uptake and distribution of the dsRNA. Additional experiments will be conducted to examine the impact of the dsRNA spray on BtMV infection transmitted by aphids.

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Exogenous application of dsRNA to control plant viruses and other pathogens and pests: it works! Now what?

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RNA interference (RNAi) is a biological process that regulates gene expression and protein synthesis by targeting specific mRNA sequences. In plants, the application of double-stranded RNA (dsRNA) from external sources can induce systemic gene silencing, leading to the suppression of specific genes in different tissues and organs. This technique has gained attention as an effective plant protection strategy against various pathogens, including viruses, fungi, and insect pests. The use of formulated dsRNA has proven to be highly effective against a wide range of pathogens and pests. When it comes to viruses, the application of formulated dsRNA has been found to significantly enhance dsRNA stability for a period of up to one month, offering robust protection against viral infections. This protection technique has been very efficient for many pathogens/pests. In the case of viruses, applying formulated dsRNA enhanced dsRNA stability for up to one month, providing efficient protection against viruses. However, the use of dsRNA in plant protection raises several safety concerns, such as off-target effects, effect on non-target organisms, epigenetics, and immune system activation. Here, we will present an update on this technique, including our recent data, and discuss safety concerns.

Recessive resistance against beet chlorosis virus is conferred by the eukaryotic translation initiation factor (iso)4E in *Beta vulgaris*

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Virus yellows (VY) disease in sugar beet is an increasing problem for European sugar beet growers and is caused by a complex of different aphid transmitted viruses, namely beet yellows virus (BYV, *Closterovirus*), beet mild yellowing virus (BMYV) and beet chlorosis virus (BChV) (both *Polerovirus*) for which a resistance is needed. Here we wanted to investigate if a resceesive resistance against the poleroviruses can be established in sugar beet. Numerous studies on the viral protein genome-linked (VPgs) of potyviruses show its' interaction with different eukaryotic translation initiation factors (eIFs) of their respective host plant to promote the viral translation. A recessive resistance to infection is achieved if the VPg-eIF interaction is lost. Initial studies in *Arabidopsis thaliana* support that this concept might be used to control the VPg-carrying poleroviruses as well. However, closely related virus species can depend on different eIFs and even further the same virus can depend on different eIFs in different hosts, making it impossible to transfer previous findings to sugar beet. Using yeast two-hybrid (YTH) and bimolecular fluorescence complementation (BiFC) assays, the physical interaction between Bv-eIF(iso)4E and the putative BChV-VPg was detected, while the VPg of BMYV was found to interact with the two isoforms Bv-eIF4E and Bv-eIF(iso)4E. These VPg-eIF interactions within the polerovirus-beet pathosystem were demonstrated to be highly specific, as single mutations within the predicted cap binding pocket of Bv-eIF(iso)4E resulted in a loss of interaction. To investigate the suitability of eIFs as a resistance resource against beet infecting poleroviruses, *B. vulgaris* plants were genome edited by CRISPR/Cas9 resulting in knockouts of different eIFs. A simultaneous knockout of the previously identified BMYV-interaction partners Bv-eIF4E and Bv-eIF(iso)4E was not achieved, but *Bv-eIF(iso)4E^{KO}* plants showed a significantly lowered BChV accumulation and decrease in infection rate from 100 % to 28.86 %, while no influence on BMYV accumulation was observed. Still, these observations support that eIFs are promising candidate genes for polerovirus resistance breeding in sugar beet.

Influence of inoculation time-point of BChV, BMYV and BYV and first results of BtMV mixed infection on sugar beet yield under field conditions

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Virus Yellows (VY) caused by beet mild yellowing virus (BMYV), beet chlorosis virus (BChV), beet yellows virus (BYV) and beet mosaic virus (BtMV) can reduce sugar beet yield up to 30-40 % (Hossain et al. 2021). The main vector in sugar beet is the green peach aphid *Myzus persicae* (*M. persicae*). The period between sugar beet emergence and canopy closure is the most critical phase for initial infection with VY. Based on trials from UK in the 1990s with an inoculation density of 100% it is assumed that there is a linear relationship between the time of infection with BMYV, BChV and BVY and sugar beet yield losses (Stevens et al. 2004, Smith and Hallsworth 1990). So far, no trials have been conducted under German growing conditions. Furthermore, there are no results of the influence of low inoculation densities in combination with different inoculation time-points on disease symptoms and sugar beet yield. In addition, little is known about the yield effect of BtMV, a member of potyviruses, well-known for synergistic effects, in single and mixed infection with other VY species under field conditions. Results from Wintermantel 2005 showed a synergistic effect of the co-infection of BtMV and BYV on disease symptoms and sugar beet yield in the greenhouse. For this reason, three completely randomized field trials, two with varying inoculation time-points and another with BtMV single and mixed infection, were conducted at two locations near Göttingen in 2023.

In the inoculation time-point trials we observed significant differences in disease severity between the different inoculation time-points within one virus species. But there are also differences between virus species. The intensity of disease severity decreased in the following order: BYV>BChV>BMYV. Across all inoculation dates, the greatest yield reductions were recorded for BYV, followed by BChV and BMYV. Within the virus species, early inoculation time-points led to significant yield losses, while the later time-points did not differ from the non-inoculated control. There is a strong negative correlation between white sugar yield losses and the calculated Area Under Disease Progress Curve (AUDPC). In the infection trial with BtMV we did not find any significant yield reduction of BtMV infection compared to a non-inoculated control. Similary, in the mixed infections with BChV and BYV, no significant yield losses or higher AUDPC were observerd compared to a single infection of each virus. Only for BMYV the co-infection with BtMV led to a higher AUDPC and higher yield losses compared to the single infection of BMYV.

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Insights into the molecular basis of beet curly top resistance in sugar beet through a transcriptomic approach at early stage of symptom development

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Curly top disease caused by geminiviruses including Beet curly top virus (BCTV) and Beet curly top Iran virus (BCTIV) is a limiting factor for sugar beet production. In recent years, climate change has increased the spread of virus vectors and disease damage in infected regions. The most economical control of BCTV in sugar beet would be through resistant cultivars though, most commercial cultivars possess only low to moderate resistance. A doubled haploid Line KDH13 showed resistance to BCTV and produced no symptoms. However, the response of Line KDH13 to BCTIV was not studied before. Here we tested the response of Line KDH13 to the BCTIV infection and compared with BCTV infection by means of inoculation with infectious clones mobilized by agrobacteria. Real-time PCR showed that both viruses replicated in the locally agroinfiltrated cotyledons. The viral DNA accumulation was lower for BCTIV compared to the BCTV infection. Systemically infected resistant plants with BCTV showed mild enation without leaf curling after 30 days. In contrast, leaf curling appeared after 12 days in the susceptible line. Further, BCTIV was not detected in resistant plants. Transcriptome analysis for the BCTV infected plants showed only 43 genes that were deregulated in line KDH13 compared to the regulation of a large number of genes (1139 gene) in the susceptible line. This work demonstrates the response of sugar beet plants to BCTV infection at both local and systemic infection and highlight the metabolic pathways/defense-related genes for their contribution towards BCTV resistance.

Comparative transcriptome analysis of virus-resistant cassava lines infected with cassava brown streak virus (CBSV)

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Cassava brown streak disease (CBSD) caused by ipomoviruses is a significant threat to cassava crops leading to the destruction of tuberous roots and high crop losses. In previous research, we identified virus-resistant cassava plants and characterized their resistance response. Resistant lines showed a complete immunity to virus infection and others restricted virus invasion to roots only. In susceptible cassava, virus replication was in all cells and tissues while in resistant lines the virus was translocated in the phloem but there was no indication for virus replication. To investigate gene expression in resistant genotypes and identify potential resistance genes, we conducted transcriptome studies using Illumina sequencing at different infection stages. We examined differential gene expression patterns across various tissues (leaves, stems and roots) at selected time points after CBSV infection and found substantial differences in gene expression between the susceptible TMS 96/0304 and the resistant DSC 260 and DSC 167 genotypes in all organs. A temporal analysis revealed significant variations in the number of differentially expressed genes (DEGs). One day after infection (1 DAI), the resistant genotypes exhibited the highest number of DEGs in stem tissue, 466 for DSC 260 and 544 for DSC 167. In contrast, the susceptible genotype had its peak at 10 DAI, with 950 DEGs in the stem. Several DEGs in the resistant genotypes were associated with proteins involved in heat shock responses, ethylene pathway, cell wall synthesis, and cell growth. The transcriptomic comparison between resistant and susceptible cassava highlights the role of gene expression regulation in resistant and susceptible response to CBSV.

Identification of the transcriptional landscape of the geminiviral infection in tomato leaves using single - cell RNA sequencing

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Geminiviruses are insect-transmitted plant viruses with circular single-stranded DNA genomes, causing devastating diseases in crops worldwide. The analyses of transcriptional changes induced by geminiviral invasion performed so far have used entire organs and most likely yielded limited information, given the low representation of infected cells in the total population and the frequent phloem-restricted nature of geminiviruses. In this work, high-throughput single-cell RNA-sequencing (scRNA-seq) was employed to profile over 30,000 cells obtained from tomato leaves infected with tomato yellow leaf curl virus (TYLCV) or mock-inoculated. By examining global gene expression patterns and well-known cell-type-specific markers, we identified seven major cell types, encompassing mesophyll, epidermis, guard cells, bundle sheath, vasculature, proliferating cells, and distinct virus-responsive cells. Through gene-signature scoring and differentially expressed genes (DEGs) analysis, we unveiled cell-type-specific responses to viral infection, highlighting notable and unique transcriptional reprogramming in vasculature and virus-responsive cells. Furthermore, we constructed a pseudotime trajectory of vasculature that elucidates the alteration of the vascular cambium caused by TYLCV. Taken together, these findings not only provide comprehensive insight into transcriptional responses to viral infection at a single-cell level but also generate a valuable resource for investigating the molecular and physiological mechanisms underlying plant-geminivirus interactions, hence potentially paving the way for the design of targeted antiviral strategies.

Exploring the geminivirus proteome: composition, intra-viral connectivity, and functionality

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Viruses are intracellular parasites with limited coding capacity that dramatically manipulate their host cell. However, how viruses can subvert the cell with only a handful of proteins still remains largely enigmatic. Geminiviruses, which can infect a large variety of crop species and lead to enormous losses in yield worldwide, have a small-sized genome, fully relying on the host molecular machinery. Members of this family therefore constitute an excellent model to comprehensively study the viral manipulation of the host cell. This project aims to unravel the emerging complexity of viral strategies illustrated in the increased coding capacity of geminiviruses and the combinatorial properties of their proteome, and obtain a comprehensive overview of the impact of this complexity on the molecular intersection between virus and infected cell with unprecedented resolution. Through a combination of in-depth analysis and exploratory approaches, we have characterized some novel genes encoding proteins localizing in specific subcellular structures and involved in the viral protein-protein interaction network, and assessed their contribution to the viral infection. We hope this project will provide novel insight into the strategies evolved by geminiviruses to maximize their interaction interface with the host, establishing a novel framework for the study of virus-host interactions.

Interplay between a polerovirus (BChV) and a closterovirus (BYV) infecting sugar beet

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Viral multi-infection is a very common phenomenon in plants that can change drastically infection parameters including transmission by insect vectors. Sugar beet is a crop frequently infected by several viruses, among them the phloem-restricted beet yellows virus (BYV, Closteroviridae) and beet chlorosis virus (BChV, Solemoviridae). Here we tested transmission of BYV and BChV by the green peach aphid (Myzus persicae) from co-infected sugar beet plants, a situation often found in the field. Co-infection decreased transmission of BChV by 50 %, but had no impact on BYV transmission. RT-qPCR analysis showed a slight decrease in accumulation of BChV in co-infected plants, indicating that lower virus accumulation might be responsible for decreased BChV transmission. Virus localisation experiments by fluorescent in situ hybridization (SABER-FISH) showed that in mono-infected plants BChV and BYV were only detected in the phloem, as expected for phloem-restricted viruses. Co-infection did not result in phloem escape of either virus. In co-infected plants, BYV and BChV colocalised in ~40 % of infected phloem cells, ~20 % of the remaining infected cells were mono-infected with BChV and ~40 % were mono-infected with BYV. BChV and BYV were detected in the phloem of the leaf midribs and the phloem of lower order vessels in mono-infected plants. In contrast, BChV was mainly restricted to the leaf midrib phloem in co-infected plants and BYV accumulated preferentially in the phloem of secondary and lower order vessels. Observation of aphids indicated that they preferred to settle and feed on lower order vessels rather than on midribs of sugar beet leaves. Thus, the differential tissue localisation of BChV in mono-infected and co-infected plants might explain the lower transmission rates of BChV from co-infected plants. Aphid feeding behaviour experiments by EPG showed that BYV mono-infection increased dramatically sap ingestion duration compared to BChV mono-infected, co-infected and healthy plants. We are currently doing transcriptomics to better understand how co-infection lowers BChV transmission, causes relocalisation of BChV and BYV.

Relieving the burden of replication: selective autophagy protects host cells against virus-induced organelle remodeling

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RNA viruses are intracellular pathogen who depend on extensive manipulation of subcellular organelles to support their replication, significantly altering their morphology. Little is known about the cellular stress response involved in coping with such stress in both plants and animals. Selective autophagy is a well-established cellular quality control mechanism that is deployed to face a multitude of environmental and developmental challenges. Versatility and selectivity towards cargoes is achieved by a multitude of selective autophagy receptor (SAR) proteins, that bridge the interaction between cargo and ATG8. Using an AP-MS screen in ss(+)RNA viruses-Arabidopsis interactions, we identify two metabolic enzyme families that bind to ATG8 in an AIM-dependent manner and undergo virus-dependent autophagic degradation. We propose that these enzymes moonlight as SARs, as they transition to a monomeric state upon virus infection and enable autophagic degradation of pro-cell death factors. Thus, we uncover a critical function of selective autophagy in promoting survival in cells undergoing active viral replication and unproductive immune response.

Poster session

No evidence for direct interaction between resistance protein Rz2 and its avirulence factor TGB1 from beet necrotic yellow vein virus in yeast-two hybrid

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Rhizomania is a devastating disease in sugar beet leading to severe losses in yield and sugar content. It is caused by beet necrotic yellow vein virus (BNYVV) which is transmitted by the soil-borne protist *Polymyxa betae*. Control relies on the resistance genes *Rz1* and *Rz2*. The resistance protein encoded by *Rz2* has been identified as classical R-protein with a coiled-coil (CC), nucleotide-binding (NB), and leucine rich repeats (LRR) domain (Capistrano-Gossmann et al., 2017). Classical R-proteins sense their corresponding avirulence factor either by direct or by indirect interaction. Recognition is mediated by the LRR domain. The triple gene block 1 (TGB1) protein of the virus has been identified as avirulence factor of Rz2. Coexpression of Rz2 and BNYVV TGB1 in the experimental host *Nicotiana benthamiana* leads to a hypersensitive response (HR) with cell death (Wetzel et al., 2021). This suggests a direct interaction between both proteins, but experimental evidence is missing. A yeast-two hybrid (Y2H) system was conducted to test for direct interaction between Rz2 and BNYVV TGB1. The results provide no evidence for a direct interaction. Expression of all proteins was confirmed by Western blot. In addition to the full-length Rz2, the separate CC and LRR domain were tested in Y2H together with BNYVV TGB1, but no direct interaction was found. To validate Y2H results *in planta*, fusion constructs for bimolecular fluorescence complementation (BiFC) were tested. Rz2 remains functional when C-terminally fused to the N-terminal part of mRFP and induces HR when coexpressed with BNYVV TGB1 fused to the C-terminal part of mRFP. Optimization is necessary to allow detection of a fluorescent signal before cell death. Subcellular localization of Rz2 and BNYVV TGB1 is studied to confirm colocalization. No detection of a direct interaction between Rz2 and BNYVV TGB1 would suggest indirect interaction with involvement of an intermediate host protein present in sugar beet and *N. benthamiana*. Proximity labeling coupled with mass spectrometry will help to identify a potential intermediate host protein as well as other unknown interaction partners.

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A Luminex MagPlex xTAG assay to distinguish infectious from non-infectious viruses

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Many plant viruses are seed transmitted and cause significant problems worldwide. Generally, heat treatment is used to inactivate viruses in seeds, but this method is a delicate equilibrium between virus inactivation and loss of seed vigour and viability. Serological or molecular tests are not useful because these cannot distinguish between intact and degraded viral RNA. Hence, there is no discrimination between infectious (’alive’) and non-infectious (‘dead’) virus. Both, in ELISA and TaqMan there is no statistical reliable difference in virus signal during heat treatment. Up until now, only an elaborate, time-consuming, and relatively insensitive bioassay can make this distinction. We developed a new method based on the Luminex MagPlex xTAG technology to determine viral infectivity of specific viruses in seeds.

Ermittlung der Genomsequenzen neuer oder unvollständig charakterisierter Potyviren aus Zierpflanzen mittel Illumina HTS

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Die Gattung Potyvirus gehört zu den umfangreichsten Virusgattungen überhaupt und aufgrund ihrer Übertragung durch Blattläuse und teilweise Saatgut sowie die häufig vegetative Vermehrung sind sie in Zierpflanzen und insbesondere Freiland-Stauden weit verbreitet. In der NCBI GenBank nuclear core collection (NC) sind derzeit von 136 die vollständigen Genomesequenzen verfügbar. Von vielen weiteren beschriebenen Potyviren gibt es jedoch nur Einträge mit partiellen Sequenzen. Dieses ist meist am 3‘-Ende des Potyvirusgenoms lokalisiert, mit einer Länge von ca. 1,5 kb, welches das CP beinhaltet. In den letzten Jahren konnten wir aus Proben verschiedener Stauden (Tradescantia, Malva, Phlox, Ranunculus) erstmals die vollständigen Genome bereits bekannter Potyviren mittels Illumina Hochdruchsatz-Sequenzierung ermitteln. Darüber hinaus wurden auch zwei bisher unbekannte Viren aus Malva und Kniphofia identifiziert. Aufgrund der niedrigen Sequenzähnlichkeiten zu Isolaten bekannter Spezies, die unterhalb der ICTV Demarkationsgrenze liegen, sind sie als Vertreter neuer Spezies der Gattung Potyvirus anzusehen.

Identifizierung eines neuen Allexivirus aus Ulme

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In virologischen Untersuchungen an Stadtbäumen haben wir ein bisher unbekanntes Ulmen-Allexivirus wurde in Blattproben eines mit chlorotischen Ringflecken, die um die Gallen der Ulmengallmilbe (*Aculus ulmicola*) auftraten entdeckt. Mittels Hochdurchsatzsequenzierung (HTS) wurde das Virus identifiziert, durch RT-PCR bestätigt und die Genomsequenz mittels RACE und inverser PCR vervollständigt.
Da Allexiviren als milbenübertragbar beschrieben sind (Kreuze et al., 2020), besteht durch das gleichzeitige Vorkommen von Virus und putativem Vektor am selben Wirt ein potentielles Risiko der Virusverbreitung. Stadtbäume sind einer Vielzahl von Stressfaktoren (wie Klimawandel und dem damit verbundenen Temperaturanstieg, Sommertrockenheit und veränderte Entwicklungen der Schädlingspopulationen) ausgesetzt, was die Vitalität reduzert und damit die Anfälligkeit der Bäume erhöht. (Bandte et al., 2022). Die an virusinfizierten Bäumen zu beobachtende Schwächung und Degeneration tritt insbesondere im Zusammenwirken mit anderen Stressfaktoren auf. Daher sollte das Auftreten von Virosen an Stadtbäumen ebenso wie andere Krankheitserreger im Kontrollmanagement berücksichtigt werden. Hierzu sind Kenntnisse über die Virosen an Stadtbäumen und die Dynamik der Viruspopulationen erforderlich. Die Identifizierung und Charakterisierung der Viren sowie die Entwicklung von optimierten Nachweismethoden sind unerlässlich (Bandte et al., 2002; Büttner et al., 2023).
Die Blätter mit den beschriebenen Symptomen wurden im Jahr 2021 von einer Goldulme (*Ulmus carpinifolia* "Wredei") in einem Wohngebiet in Hamburg entnommen und zusammen mit fünf weiteren Blattproben von Gehölzen sequenziert. Aus dem Blattmaterial wurde die Gesamt-RNA extrahiert und die rRNA mittels RiboMinus Plant Kit for RNA-Seq (Invitrogen) abgereichert. Für die cDNA-Synthese wurden das Maxima H Minus Double-stranded cDNA synthesis Kit (Thermo Fisher Scientific) und random hexamer Primer eingesetzt. Die Sequenzierung wurde von BaseClear (Leiden, Niederlande) auf einem Illumina NovaSeq 6000 System durchgeführt. Die Datenanalyse erfolge mittels Geneious Prime (Auckland, Neuseeland). Die provisorische Zuordnung zu den Allexiviren erfolgte mittels phylogenetischer Analyse der Replikase- und Nukleokapsid-kodierenden Regionen. Ein erstes Screening wurde an Blattproben von Berliner Ulmen durchgeführt. Daten zum aktuellen Wissensstand bisher unbekannten Allexivirus aus Ulme werden vorgestellt und diskutiert.

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Highly curated and reliable E-probes for detection of hop pathogens in unassembled HTS datasets.

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High-throughput sequencing (HTS) has revolutionized viral detection by demonstrating the ability to detect known and unknown viruses infecting plants. Bioinformatics expertise, programming skills, and access to high-capacity computers are essential for HTS data analysis. A bioinformatic detection system that addresses these HTS constraints and performs searches on non-normalized HTS data was used; the E-probe Diagnostic Nucleic acid Analysis (EDNA), reported in (Stobbe et al., 2013), allows the design of electronic probes (e-probes) to detect pathogens in raw HTS metagenomic outputs eliminating the assembly steps of HTS. EDNA was used to design e-probes for two viroids and three viruses that infect the hop plant (Humulus lupulus L.) and were identified in metagenomic datasets in a study profiling the German hop virome. The e-probes were designed to cover the genomic sequences of the targeted viruses/viroids using the EDNA theoretical framework updated in 2021 to operate in a Windows environment named Microbe Finder (MiFi®) (Espindola and Cardwell, 2021). The designed e-probes have undergone a rigorous curation process to minimize the likelihood of false negatives and false positives. The curated e-probes were validated in silico using an HTS metagenome simulator (MetaSim) to predict their specificity and limit of detection. Additionally, an in vitro validation followed by constructing an artificial positive control (APC) that carried all curated e-probes cloned into the APC. Serial dilutions made from the APC stock plasmid were HTS and the detection limit of the e-probes of the serially diluted targets was determined. The developed e-probes demonstrated robustness, sensitivity, and efficacy when applied to HTS datasets previously analyzed using other bioinformatics tools (Geneious Prime and Virtool). The EDNA-MiFi® e-probes developed in this study enable the direct detection of targeted hop viruses/viroids in HTS data, making EDNA-MiFi® for hop a valuable tool for surveillance and production of certified virus-free plant material. It is also reduces the need for bioinformatics expertise or high-performance computing.

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Genetic diversity analyses of ash shoestring-associated virus (ASaV) in Germany

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Ash shoestring-associated virus (ASaV) has been found in *Fraxinus excelsior* and *F. ornus* trees, causing virus-typical leaf symptoms such as shoestring, chlorotic spots, line patterns, and deformations. ASaV along with various other biotic and abiotic factors, contribute to the decline of *Fraxinus* spp. (Büttner et al., 2023, Gaskin et al., 2021). ASaV belongs to the genus *Emaravirus* (Order *Bunyavirales*; Family *Fimoviridae*) (Gaskin et al., 2021). Emaraviruses can infect various herbaceous and woody plants. They are enveloped and have a multipartite, single-stranded, negative-sense RNA genome with conserved and complementary sequences at the 5′ and 3′ termini of each genome segment (Kormelink et al., 2011, Mielke-Ehret et al., 2012, Elbeaino et al., 2018, Büttner et al., 2023). The ASaV genome consists of five RNAs, each encoding a single open reading frame (ORF). The virus RNA-directed RNA polymerase (RdRp), the glycoprotein precursor (GPP), the viral nucleocapsid protein (N), and the movement protein (MP) are encoded by RNA1-RNA4, respectively. A 26 kDa protein of unknown function (P26) is encoded by RNA5 (Gaskin et al., 2021, Rehanek et al., 2022). Little is known about the genetic variation of ASaV. The Population of RNA viruses with a segmented genome is expected to exhibit high genetic diversity due to the generation of immense population size of viruses, high mutation rates, and the occurrence of recombination and reassortment events within their genomes (Moya et al., 2004). Viral population genetic diversity has a fundamental role in populations adapting to changing selection pressures. The characterization of the genetic variation of the viral population will resolve ambiguities about the epidemiology and the evolutionary history of the virus. We are reporting sequence comparisons of ASaV genome variants determined from different nearly complete RNA segments from *Fraxinus* species and different geographical regions in Germany. Knowledge and information about viral epidemiology and evolution can lead to the development of efficient and durable disease management.

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Risk of hop viroids in citrus-based plant-strengthening products

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Germany is renowned as the world's largest producer of bitter hops, known for their exceptional quality. However, hops (*Humulus lupulus*) are vulnerable to various abiotic and biotic stressors, including the citrus bark cracking viroid (CBCVd), which originated from citrus plants. This viroid can cause severe symptoms and substantial yield losses. There has been an increasing trend towards organic agriculture worldwide, including hop cultivation. Further, driven by consumer preferences and strict EU regulations, there have been restrictions or bans on synthetic plant-protection products due to concerns over chemical residues and environmental harm. This shift has prompted the search for alternative products to strengthen or protect crops. For hop cultivation, citrus-based solutions have been developed and marketed as plant-strengthening products. However, these may carry the unknown risk of introducing viroids like CBCVd. This study is a starting point for assessing the risk associated with using citrus-based plant-strengthening products. We developed a citrus oil RNA extraction protocol and evaluated three citrus-oil-based plant-strengthening products for CBCVd, citrus exocortis viroid (CEVd), and hop stunt viroid (HSVd) using RT-PCR. We confirmed through spiking experiments that RT-PCR viroid detection is possible in different oil-viroid mixtures (96:4;4:96). Therefore, we then extracted citrus oil freshly from infected fruit peels using a cold oil press, simulating the production process for plant-strengthening products. The resulting oils were tested for CBCVd, CEVd, and HSVd. RT-PCR analysis revealed no viroid transmission from the fruits to the oil, leading us to conclude that these oils likely pose a low risk of containing hop pathogenic viroids. However, the presence of viroids at concentrations below the detection limit cannot be completely excluded. This study represents a significant initial step towards developing safe, sustainable, and eco-friendly hop products.

Genetic Diversity and Population Structure of the novel Aspen Mosaic-Associated Virus (AsMaV) in Populus Trees across Finland and Sweden

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A novel *Emaravirus*, named aspen mosaic-associated virus (AsMaV), has been identified as the associated agent of mosaic disease in *Populus tremula* (von Baegen et al., 2020; Rehanek et al., 2023; Büttner et al., 2023). The complete genome of AsMaV comprises five negative-sense single-stranded RNA (-ssRNA) molecules. Notably, RNA1 (7.1 kb) encodes the viral RNA- dependent RNA polymerase (RdRP, 268.2 kDa), while RNA2 (2.3 kb), RNA3 (1.6 kb), RNA4 (1.6 kb), and RNA5 (1.3 kb) encode the glycoprotein precursor (GPP, 73.5 kDa), viral nucleocapsid protein (N, 35.6 kDa), a putative movement protein (MP, 41.0 kDa), and a protein of unknown function (P28, 28.1 kDa), respectively (von Bargen 2020, Nourinejhad Zarghani 2023). To investigate genetic diversity and population genetic parameters, various regions of the virus genome were targeted. Full-length AsMaV-RNA3, -RNA4, and partial -RNA1 were amplified using specific primer pairs through RT-PCR. Subsequent analyses included RT-PCR- RFLP, and selected variants underwent sequencing. Results indicated a conserved genome across AsMaV variants; however, isolates from Finland and Sweden formed distinct phylogenetic groups, suggesting the existence of separate populations. Furthermore, discrimination among AsMaV variants was more effective based on RNA3 than RNA4 or partial RNA1. This study provides valuable insights into the genetic makeup and population dynamics of AsMaV, paving the way for a comprehensive understanding of its epidemiology and potential management strategies.

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Mycoviruses suppress production of the mycotoxin deoxynivalenol by Fusarium graminearum

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RNA-viruses of the genera Ambivirus, Mitovirus, Sclerotimonavirus, and Partitivirus were found in a single isolate of Fusarium graminearum. The genomes of the mitovirus, sclerotimonavirus, and partitivirus were assigned to previously described viruses, whereas the ambivirus genome putatively represents a new species, named Fusarium graminearum ambivirus 1 (FgAV1). To investigate the effect of mycoviruses on the fungal phenotype and biological properties, spontaneous loss of mycoviruses during meiosis and transmission of mycoviruses into a new strain by anastomosis were used to obtain isogenic F. graminearum strains with and without mycoviruses. Notable effects observed in mycovirus-harboring strains were (i) suppression of the synthesis of trichothecene mycotoxins and their precursor trichodiene, (ii) suppression of the synthesis of the defense compound aurofusarin, (iii) stimulation of the emission of 2-methyl-1-butanol and 3-methyl-1-butanol, and (iv) increased attractiveness of fungal mycelia for fungivorous collembolans. Increased attractiveness of mycovirus-infected filamentous fungi to animal predators opens new perspectives on the ecological implications of virus infections in fungi.

Early transcriptomic response in sugar beet leaf tissue following aphid-mediated infection with beet yellows virus

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Beet yellows virus (BYV), type member of the genus Closterovirus in the family Closteroviridae, is considered the most devastating pathogen of virus yellows disease in sugar beet causing yield losses of up to 49 % depending on environmental conditions and timing of the infection (Smith and Hallsworth, 1990). The molecular mechanism by which BYV reduces the root yield and sugar content is still unclear, however, yellowing is known to be initiated by the virus by de-regulating chlorophyll biosynthesis-related genes. We studied the mRNA de-regulation profile in a susceptible sugar beet cultivar following aphid-mediated BYV inoculation on mature leaves at three early infection stages [6, 24 and 72 hours post inoculation (hpi)] using RNAseq. In total, 588 differentially expressed genes (DEGs), 370 of which were up regulated and 218 down-regulated, were identified within the three time intervals, when individually compared to mock-aphid inoculated leaf samples of the same time point, eliminating the effect of aphid feeding itself. We unraveled that de-regulation processes are started in the plant already at a very early time-point (6 hpi). By using MapMan and Mercator4-based gene annotation for sugar beet transcript categorization, early differential gene expression identified importance of the categories ″phytohormone action″, ″cell wall organization″ and ″solute transport″. The virus was detectable via RT-PCR at the initial site of infection three days after aphid inoculation. Among others, at this stage of infection, chloroplast-related genes were already down regulated what was also validated by RT-qPCR analysis of biological control samples. These results provide first insights into which genes are targeted early after infection and improve the understanding of the plant-virus interaction.

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Integrated microscopy investigations aimed at elucidating interactions between viruses and their host within the plant nucleus

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Transcription, replication and encapsidation of begomoviruses, as with all ssDNA viruses, take place in the nucleus. A temporal and spatial coordination of these essential processes in the nuclear phase of the infection cycle is no longer a hypothesis, but has not yet been described in detail. This study therefore first focuses on the changes in the localization of specific nuclear marker proteins in response to a begomoviral infection. The results show temporally altered localization of these nuclear marker proteins during viral infection, suggesting organization of membraneless nuclear microcompartments probably essential for infection.

Psammotettix alienus as potential vector of pathogenic phytoplasmas

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The study investigates recently observed *Wheat dwarf virus* (WDV)-like symptoms in wheat and barley plants that occurred after inoculation with *Psammotettix alienus* leafhoppers from our rearing. Symptomatic plants show a dwarfted witches‘ broom growth, narrowed leaves with white or yellow discolorations, formation of sticky secretion droplets on the leaf surface and sterile ears. Based on the symptoms a viral causal agent was initially suspected but DNA metabarcoding of 16S rRNA confirmed the presence of phytoplasmas in *P. alienus* and infected cereal samples. Phytoplasmas are obligate intracellular bacteria of the phloem. Several phytoplasma diseases in cereals mainly transmitted by leafhoppers of the family *Cicadellidae* are reported from Asia, Afrika, and European countries. First results show highest similarity to *Candidatus Phytoplasma asteris* and *Campsis grandiflora phytoplasma* strains. A rapid molecular diagnostic is possible by using a universal phytoplasma nested PCR. Tetracycline treatment decreases symptom severity. A further verification of the detected phytoplasma is planned by microscopic investigations and sequencing approach. We like to collect samples from (regional) cereal fields to monitor natural phytoplasma distribution. Changing climate conditions may lead to increased phytoplasma reports in Germany.

Ornamentals matter: Impact of ornamentals for the One Health-concept and as virus reservoir in the Anthropocene

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Currently 374.000 plant species are known and 10% are used. Metagenomics revealed viruses in asymptomatic plants. This virus reservoir can be a threat for cultured plants. So far, 2.000 plant viruses are classified. The majority of plant viruses still has to be discovered.

A risk assessment study of the virome of the tuberous crop Mashua (*Tropaeolum tuberosum*)

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In 2020, a High Throughput Sequencing (HTS) study of six tuberous crops originating from outside of Europe, including Mashua (Tropaeolum tuberosum), Oca (Oxalis tuberosa), Anredera cordifolia and Ulluco (Ullucus tuberosus), showed the presence of a considerable number of viruses belonging to different agriculturally important virus groups. These findings were confirmed by similar studies performed in Belgium and the UK within the Euphresco PRONC project. In a follow-up study in 2022 and 2023, a more detailed HTS-analysis was performed on Mashua tubers from 5 different cultivars obtained through international web shops. The presence of the earlier identified viruses was again confirmed with all samples contained multiple viruses., belonging to at least 8 different virus genera. The majority of these viruses were 'new' viruses, not described before. For four of the identified viruses, biologial assays were performed to assess their potential risks on plants including agricultural important crops like tomato, pepper and potato. The identification of the viruses in the virome of Mashua and the results of the biological risk assessment will be discussed.

Studies on the N-terminal signal peptide of celery latent virus (CeLV)

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Celery latent virus (CeLV), a member of the genus Celavirus in the family Potyviridae, shows some unique features compared to other viruses in this family. Especially remarkable is an N-terminal localized signal peptide (SP), which has been shown to guide proteins to the endoplasmic reticulum. To our knowledge, this has not yet been described for a Potyviridae member. Another signal peptide in plant viruses was predicted for bean necrotic mosaic virus (BeNMV, Tospovirus). It is located at the N-terminus of the GN/GC glycoprotein precursor.

The aim of this study was to investigate whether the CeLV-SP is essential for infection in plants and if the BeNMV-SP can replace it. First, mutations were introduced and analysed in co-localization studies for subcellular localization and second integrated analogously into an infectious full-length clone. Infection of plants was carried out by Agrobacterium- mediated transformation with subsequent RT-PCR detection. In the positive case, a 5'-RACE was performed with subsequent sequencing. Initial results showed that deleting the CeLV-SP, replacing it with the BeNMV-SP or certain mutations of the CeLV SP lead to rarely detectable or no infection in plants. In the case of positive detections, only revertants could be identified. Other mutations had no influence on infectivity and were sequenced from the plants without alteration.