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Gesellschaft e.V.

55th meeting of the DPG Working Group
"VIRUSKRANKHEITEN DER PFLANZEN"
March 20th and March 21th 2023

at

Julius-Kühn-Institut, Berlin

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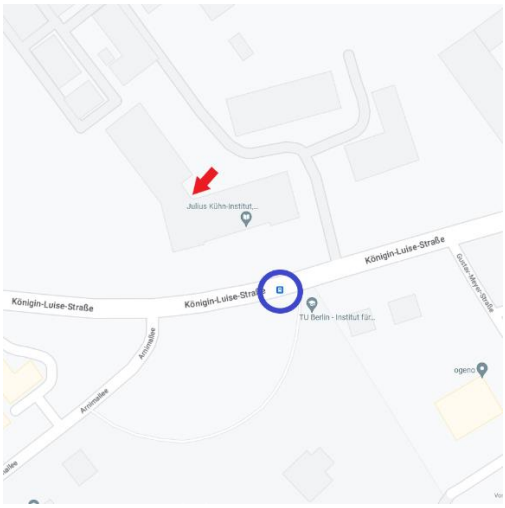
DPG-working group leaders: Dr. Annette Niehl & Dr. Björn Krenz

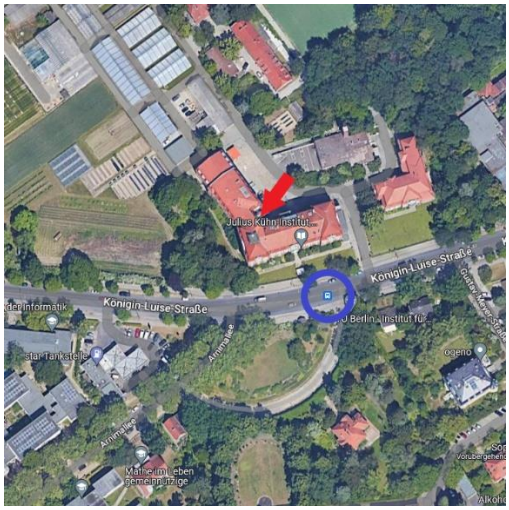
Organisation & host: Prof. Dr. Carmen Büttner & team

Dear colleagues,

We and the team of Prof. Dr. Carmen Büttner are welcoming you to the 55th meeting of the DPG Working Group " Viruskrankheiten der Pflanzen" in Berlin on Monday, March 20th 2023 and Tuesday March 21th 2023.

The venue is the Julius Kühn Institute:
Julius Kühn Institut, Königin-Luise-Strasse 19, 14195 Berlin.
Room A 300 (3 floor)
Please use the entrance in the "inner bend" of the L-shaped JKI building (see also pictures).





We look forward to a meeting that will benefit from numerous and diverse contributions, discussions and questions. The 2023 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 30 € to cover the catering (the dinner & 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 (BigBlueButton):

https://lecture.julius-kuehn.de/b/ebe-fh0-tig-smh

This link is reserved for conference participants.

With kind regards,
Annette Niehl & Björn Krenz

Our keynote speakers

Richard Kormelink is Associate Professor at the Laboratory of Virology, Wageningen University. He obtained his PhD degree on the study of the structure and expression of plant-infecting bunyavirus genomes in 1994. Ever since, he is interested in diseases and the cytopathology of segmented, negative-strand RNA viruses of plants and vertebrates (Bunyaviruses, Influenzaviruses and Arenaviruses, Tenuiviruses) and in specific how structurally and genetically similar plant- and animal-infecting (bunya)viruses cope with defence responses in hosts belonging to different kingdoms and their insect vectors. His current focus is on antiviral RNAi and viral counter defence by RNA silencing suppressor proteins, dominant R (NLR) genes, viral effectors and epigenetic mechanisms controlling geminiviruses and other DNA elements. One of the objectives that receives quite some attention at this moment aims to unravel the mode of action of the Ty-1 resistance gene, an a-typical single dominant resistance gene that confers resistance to DNA geminiviruses via enhancement of the transcriptional gene silencing response.

Recent highlights:

1. Siskos, L., Antoniou, M., Riado, J., Enciso, M., Garcia, C., Liberti, D., (Tahirov, T.H.), Visser, R.G.F., Kormelink, R., Bai, Y. and Schouten, H.J. (2023). DNA primase large subunit is an essential plant gene for geminiviruses, putatively priming viral ss-DNA replication. **Frontiers in Plant Sciences**, DOI: 10.3389/fpls.2023.1130723.
2. Van Grinsven, I.L., Marin, E.C., Petrescu, A.J. and Kormelink, R. (2022). Tsw -A case study on structure-function puzzles in plant NLRs with unusually large LRR domains. **Frontiers in Plant Sciences 13**, 983693.
3. Xu, M., Mazur, M.J., Gulickx, N., Hong, H., Overmars, H., Tao, X. and Kormelink, R. (2022). Bunyaviral N proteins localize at RNA Processing Bodies and Stress granules: The enigma of cytoplasmic sources of Capped RNA for Cap snatching. **Viruses 14**, 1679-1726.

Fritz Kragler holds a permanent Group Leader position at the Max-Planck-Institute of Molecular Plant Physiology. He obtained his PhD degree in Genetics/Biochemistry at the University of Vienna on the study of protein transport into peroxisomes. Since his Postdoc time at the University of California, Davis he works on the intercellular and long-distance transport of viral and plant endogenous macromolecules. His current work focuses mainly on long-distance mRNA transport in plants and the mRNA motifs, secondary modifications, and RNA-binding proteins necessary to mediate mRNA transport between tissues.

Recent highlights:

Yang L, Machin F, Wang S, Saplaoura E, Kragler F. 2023. Heritable transgene-free genome editing in plants by grafting of wild-type shoots to transgenic donor rootstocks. Nat Biotechnol.

Yang L, Zhou Y, Wang S, Xu Y, Ostendorp S, Tomkins M, Kehr J, Morris RJ, Kragler F. 2023. Noncell-autonomous HSC70.1 chaperone displays homeostatic feedback regulation by binding its own mRNA. New Phytol 237(6): 2404-2421.

Kehr J, Morris RJ, Kragler F. 2022. Long-Distance Transported RNAs: From Identity to Function. Annu Rev Plant Biol 73: 457-474.

Yang L, Perrera V, Saplaoura E, Apelt F, Bahin M, Kramdi A, Olas J, Mueller-Roeber B, Sokolowska E, Zhang W, et al. 2019. m(5)C Methylation Guides Systemic Transport of Messenger RNA over Graft Junctions in Plants. Curr Biol 29(15): 2465-2476 e2465.

PROGRAMME

Monday, 20. March 2023	
11:00 – 12:30	**Registration with snacks, soup and drinks**
12:30 – 12:40	**Welcome & conference information** *Annette Niehl & Björn Krenz*
12:40 – 12:50	**Introduction of DPG & Junge DPG (please become a member)** *Annette Niehl & Björn Krenz*
12:50 – 14:55	**Section I: "from -omics to plant virus resistance" – Carmen Büttner & Björn Krenz**
12:50 – 13:40	**Keynote** **On the stories behind (a)typical dominant and recessive resistance genes against geminiviruses** *Richard Kormelink*
13:40 – 13:55	**Approaches and challenges in the use of high-throughput sequencing for virus discovery in plant materials from various provenance** *Paolo Margaria*
13:55 – 14:10	**A glimpse into the German hop virome** *Ali Pasha*
14:10 – 14:25	**Fast in silico pathogen detection in high-throughput sequencing demonstrated for hop pathogenic viroids** *Michael Hagemann*
14:25 – 14:40	**Laser-assisted microdissection combined with RNAseq to investigate early plant-virus interaction. A case study of Turnip mosaic virus in Arabidopsis thaliana plants** *Khalid Amari*
14:40 – 14:55	**Transcriptome analysis of cassava brown streak virus (CBSV) infected cassava** *Jessica Lilienthal*
14:55 – 15:20	**Coffee & tea break**
15:20 – 17:20	**Section II: "beets & more" – Christina Wege**
15:20 – 15:35	**Characterization of the interaction between the R protein Rz2 from Beta vulgaris with the triple gene block protein 1 of different virus** *Sebastian Liebe*
15:35– 15:50	**Manipulating betalain biosynthesis using beet mosaic virus mediated expression of BvMYB1 transcription factor allows visual virus tracking in Beta vulgaris** *Lukas Rollwage*
16:20 – 16:35	**Co-infection with other sugar beet viruses modifies the transmission and localization of beet yellows virus.** *Souheyla Khechma*
16:35 – 16:50	**Host range, molecular and ultrastructural analyses of Asparagus virus 1 isolated from Asparagus officinalis L.** *Katja Richert-Pöggeler*
16:50 – 17:05	**Comparison of the prevalence of ASaV-infected flowering ash (F. ornus) trees in two Germany Metropoles** *Kira Köpke*
17:05 – 17:20	**Environmental and genetic drift of the wheat-polymyxa-furovirus interaction** *Kevin Gauthier*

17:20 – 17:30	**short break (10min)**
17:30 – 18:15	**Poster flash talks (3min each) - Björn Krenz & Annette Niehl** 1. Investigations on the protease activity in the N- and C-terminus of celery latent virus (CeLV) by in vitro translation *Svenja Lindenau* 2. Studies on the presence of a functional PIPO (pretty interesting Potyviridae open reading frame) as a functional component of the polyprotein of Celery latent virus (CeLV) *Hanna Rose* 3. JSBWMV movement protein - identification and characterization of an interacting host-plant RNA-binding protein *Claudia Strauch* 4. Unravelling the nuclear manipulation of geminiviruses *Christoph Sicking* *5.* Lipidomics of AbMV infected nuclei *Ina Schmidt* 6. Mycoviruses in Fusarium graminearum –Host transition of Fusarium poae virus 1 *Simon Schiwek* *7.* Investigations on the virus status of mother trees and seedlings of common ash (*Fraxinus excelsior*) from the Melzower Forest - A study within the FraxVir project *Linda Frey* 8. Gu2mCBJpAxMolecular detection of beet mild yellowing virus (BMYV), beet chlorosis virus (BChV), beet yellows virus (BYV) and beet mosaic virus (BtMV) in Myzus persicae by Real-Time PCR *Simon Borgolte* 9. Vector monitoring of flavescence dorée phytoplasmas, Xylella fastidiosa and regulated non-quarantine pests in fruit crops and viticulture *Christine Seinsche* 10. Searching for a mild isolate to use in cross-protection studies for ToBRFV *Mareike Rohde* *11. The use of Tagetes minuta to control grapevine fanleaf virus (GFLV)* *Sabine Oettl* *12. Seasonal fluctuation of cherry viruses in South Tyrol (Italy)* *Sabine Oettl* 13. Investigations into the virus status of blueberries in Germany with a special focus on the so called "off type" *Wulf Menzel*
19:30	**Conference dinner**

Tuesday, 21. March 2023	
08:25 – 08:30	**Welcome & conference information** *Annette Niehl & Björn Krenz*
08:30 – 10:20	**Section III: "It's all about RNA" – Khalid Amari**
08:30 – 08:45	**Manipulation of the plant RNA splicing machinery by geminiviruses** *Delphine Pott*
08:45 – 09:00	**Evidence for the splicing of complementary-sense transcripts of Beet curly top Iran virus** *Omid Eini*
09:00 – 09:05	**Analyzing splicing as a novel strategy of bipartite begomoviruses to increase their coding capacity** *Jessica Gehrke*
09:05 – 09:55	**keynote (40 + 10 min)** **Lost in translation? The long-distance travel of messenger RNAs** *Fritz Kragler*
5min break	**Section IV: "DNA viruses" - Richard Kormelink**
10:00 – 10:15	**A viral peptide as a trigger for the relocalization of a viral effector** *Laura Medina-Puche*
10:15 – 10:30	**A novel assay demonstrates a possible mode of action for Beet Curly Top Iran Virus V2 as a suppressor of transcriptional gene silencing** *Arvid Hanke*
10:30 – 10:45	**A plant virus causes symptoms through the deployment of a host-mimicking protein domain to attract the insect vector** *Man Gao*
10:45 – 11:45	**Coffee & tea break and poster session (60min)**
11:45 – 12:55	**Section V: "technics & fields" - Wilhelm Jelkmann & Susanne van Bargen**
11:45 – 11:55	**Development of serological test methods for the detection of novel emaraviruses in oak and ash trees** *Marius Rehanek*
11:55 – 12:05	**Testing of German chickpeas for various viral infections** *Christoph Kartheuser*
12:05 – 12:15	**Construction of a turnip yellows virus (TuYV) cDNA full-length clone of an isolate originating from sugar beet to study its host plant spectrum** *Roxanna Hossain*
12:15 – 12:25	**Tomato brown rugose fruit virus in the Netherlands: Epidemiological insights with Nextstrain** *Pier deKönig*
12:25 – 12:35	**Potential risk of Tomato brown rugose fruit virus carry-over by surfaces in a commercial tomato greenhouse** *Jens Ehlers*
12:35 – 12:45	**Tomato brown rugose fruit virus contaminated clothing of greenhouse employees is a challenge for cleaning** *Jens Ehlers*
12:45 – 12:55	**Virusdiagnostik für den amtlichen Pflanzenschutzdienst in Brandenburg** *Marko Riedel*

12:55 – 13:15	**short break (20min)**
13:15 – 14:00	**Online flash talks (7min each) - Björn Krenz & Annette Niehl** • Cassava brown streak HAM1 gene and its role in the resistant response in cassava Samar Sheat • Dynamic subcellular distribution of begomoviral nuclear shuttle and movement proteins Tatjana Kleinow • Emerging multifactorial complexity at the geminivirus-host interface Yu Zhou • Investigating the role of the chromatin regulator LHP1 in defense against geminiviruses and its interplay with the geminivirus-encoded C3 protein Zhihao Jiang • Unravelling the molecular mechanisms underlying the replication of geminiviruses Chaonan Shi • Uncovering novel virulence strategies deployed by tomato yellow leaf curl virus Huang Tan
14:00 -	**Farewell & honors** *Annette Niehl & Björn Krenz*

ABSTRACTS

Monday, 22. March 2023
Section I

Keynote

On the stories behind (a)typical dominant and recessive resistance genes against Geminiviruses

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Tomato yellow leaf curl geminivirus (TYLCV) belongs to the most devastating plant viruses worldwide. One way to combat this virus is by resistance breeding. From the list of available resistance genes for introgression breeding in tomato against TYLCV, Ty-1 to -6, several have been mapped and cloned in the past decade. Whereas *Ty-2* presents a typical dominant resistance gene of the "nucleotide-binding site Leucine-rich repeats" (NLR) class and is triggered by the Rep/C1 protein, *ty-5* presents a recessive resistance gene coding for *pelota*, an mRNA (translation) surveillance factor. On the other hand, *Ty-1* and its *Ty-3* allele present an a-typical dominant resistance gene, coding for an RNA-dependent RNA polymerase (RDR) of the gamma class and implicated in antiviral RNAi. While dominant resistance genes of the NLR class are the most commonly deployed genes for resistance breeding, they are pathogen specific and can easily be broken by the emergence of a new pathogen variant containing only one single point mutation in the pathogen effector. This presents a major drawback of using NLR resistance genes. In contrast, atypical dominant and recessive resistance genes often provide more durable and broad resistance, and for that reason receive a growing interest. The stories and underlying resistance mechanism behind Ty-1 and DNA primase Large subunit (*PriL*), another recently identified gene in melon accessions correlated to a recessive resistance against Tomato leaf curl New Delhi virus (ToLCNDV), will be presented in support of their exploitation and implementation towards more durable resistance / disease management strategies. First of all, to combat geminiviruses.

Approaches and challenges in the use of high-throughput sequencing for virus discovery in plant materials from various provenance

Margaria P

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High-throughput sequencing (HTS) technologies are transforming our means to detect pathogens and perform disease diagnosis. Their application to detect and characterize (novel) plant pathogens and pests has been growing steadily in the last years and increased implementation for routine tests in plant pest diagnostics is foreseen. Nevertheless, challenges in the use of HTS technologies remain, covering all steps of the workflow, from sample/library preparation to sequencing technology and bioinformatics analysis of the generated data. The sensitivity to these aspects is proved by current efforts to define guidelines and recommendations for the adoption and reliable use of HTS in diagnostic laboratories. We have implemented HTS-technologies as a fundamental tool for rapid virus discovery and we routinely employ them for the screening of plant materials from various provenance, to provide quick pathogen identification in diseased material and support the safe exchange of germplasm resources. Approaches used and challenges encountered at various steps will be presented.

A glimpse into the German hop virome

Pasha A, Ziebell H

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Germany is the second-biggest hop producer in the world, with more than 20,600 hectares of hop-cultivated area. Many pests and pathogens threaten to hop production. These can cause severe reductions in hop yield and quality.

In summer 2019, citrus bark cracking viroid (CBCVd) was reported for the first time in Germany. CBCVd is a pathogen of citrus plants that causes mild and often tolerated infections of different Citrus species, whereas it causes severe disease in hop plants.

The project "HopfenViroid" is addressing practical and scientific questions regarding CBCVd. As a part of this project, high-throughput sequencing (HTS) is being applied to investigate the viro-diversity in different German hop-growing sites. In 2021, we started with a pilot study targeting three fields in Hallertau (southern Germany), where CBCVd was previously detected. The samples were collected from hop, non-hop inside the field, and non-hop outside the field. Samples were pooled, double-stranded RNAs were extracted as a viral and viroid enrichment approach (Gaafar and Ziebell 2020), and followed by Illumina sequencing. The bioinformatic analysis showed that all identified viruses and viroids in hops across the three fields were previously described as hop pathogens. In 2022, this study was extended to cover three different hop-growing sites in Germany. The sampling and pooling strategies used for the pilot study were followed in 2022. The HTS-data analysis revealed common hop viruses and a viroid infecting the German hops. CBCVd was identified in hops in one site. A non-hop virus was identified in hops in three fields across the targeted sites. This study will be continued in 2023 to obtain a comprehensive understanding of the viro-diversity in German hops.

REFERENCES:

Gaafar YZA, Ziebell H (2020). Comparative study on three viral enrichment approaches based on RNA extraction for plant virus/viroid detection using high-throughput sequencing. PLoS ONE 15(8): e0237951.

Fast in silico pathogen detection in high-throughput sequencing demonstrated for hop pathogenic viroids

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The number of regulated plant pathogens is constantly rising and changing due to global trade and climate change. Furthermore, modern highly sensitive methods such as high-throughput sequencing (HTS) make it possible to detect several pathogens in parallel in a single analysis. However, the bioinformatic analysis of high-throughput sequencing (HTS) requires the user to have computer coding skills and, considering the file size and complicated algorithms, access to high-performance computing. To overcome those constraints, the user-friendly Microbe Finder (MiFi®) web-based tools has been developed at the Oklahoma State University (Espindola & Cardwell, 2021). Here, we compare the e-probes based MiFi tool, with standard HTS analysis pipelines and (q)PCR data. We compare the sensitivity, discuss limitations, and estimate the time and cost investments per analysis. Our study object is hop (*Humulus lupulus* L.) either infected or uninfected with the citrus bark cracking viroid (CBCVd). CBCVd is leading to production losses for the European hop industries, particularly in Slovenia and Germany.

The results show that the curated CBCVd e-probes are highly specific and indeed qualify as a quick method for pathogen detection from Illumina sequencing data. Furthermore, our e-probes did work with data derived from the sequencing of total RNA, double-stranded RNA, or small RNA. A constraint of the MiFi® tool might be that the raw data needs to be uploaded to non-European servers for the analysis and that the cloud storage is limited. Therefore, we also show alternative but computationally more demanding fast data analysis methods. In any case, using fast *in silico* detection pipelines is a promising approach once established for several pathogens and considering that sequencing cost might further drop in the future.

REFERENCE

Espindola AS; Cardwell KF (2021). Microbe finder (Mifi®): Implementation of an interactive pathogen detection tool in metagenomic sequence data. *Plants* **10**, 1–14.

Laser-assisted microdissection combined with RNAseq to investigate early plant-virus interaction. A case study of *Turnip mosaic virus* in *Arabidopsis thaliana* plants.

Khalid Amari[1] and Björn Krenz[2]

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Turnip mosaic virus (TuMV) is a member of the genus *Potyvirus* (family *Potyviridae*) belonging to the picorna-like supergroup. TuMV genome is a single-stranded positive-sense RNA, encoding for a large polyprotein which is subsequently cleaved by three viral-encoded proteinases. TuMV infection causes serious damage in several plants especially cruciferous ones. Success or failure of the establishment of an infection depends on the outcome of early interactions between viruses and plants. Therefore, it is of great interest to investigate the early plant-virus interplay and to decipher the molecular mechanisms governing this interaction. Here we used laser-assisted microdissection (LMD) combined with the highly cost-effective method Smart-3SEQ technology to determine the transcriptomic profile in TuMV-infected Arabidopsis plants after 2 dpi. Even though the amount of RNA input was low due to the use of only a few plant cells collected by LMD, the Smart-3SEQ RNAseq yielded enough reads number with good quality, consistent with previous studies [1]. Gene ontology and pathway enrichment analysis were conducted to functionally characterize differentially expressed genes (DEGs). Our results show that TuMV infection modulates early host genes expression. Several genes involved in plant response to stress in general and to viruses in particular were upregulated and genes involved in photosynthesis and translation were downregulated.

REFERENCE

J. W. Foley *et al.*, "Gene expression profiling of single cells from archival tissue with laser-capture microdissection and Smart-3SEQ.," *Genome Res.*, 2019, doi: 10.1101/207340.

Transcriptome analysis of cassava brown streak virus (CBSV) infected cassava

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Cassava brown streak disease (CBSD) is a significant threat to cassava crops caused by ipomoviruses, which lead to the destruction of tuberous roots and ultimately crop loss. In previous research, we identified virus-resistant cassava plants and characterized their resistance response. Resistant lines showed complete immunity to virus infection, whereas virus spread was observed in roots of other lines. However, all aboveground organs remained symptom-free and virus-free. Localization studies revealed that CBSV replicate throughout tissues in susceptible lines but was detected exclusively in the phloem of resistant plants. To investigate the tissue-specific gene expression of the resistant lines and to identify potential resistance genes, we performe transcriptome studies. For this purpose, leaf samples of long-term infected, susceptible and resistant lines were analyzed by Illumina sequencing. The results showed significant differences in gene expression of proteins assigned to domains of photosynthesis, jasmonic acids, salicylic acids, and autophosphorylation. Subsequent transcriptome studies will analyse multiple time points directly after CBSV infection of leaf, stem and root material. In addition, phloem cells of the stem will be enriched by laser dissection microscopy (LDM) to further investigate gene expression in the phloem.

Characterization of the interaction between the R protein *Rz2* from *Beta vulgaris* with the triple gene block protein 1 of different virus species

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The beet necrotic yellow vein virus (BNYVV, genus *Benyvirus*) causes the economically important rhizomania disease in sugarbeet. The virus is controlled by two separate dominant resistance genes called *Rz1* and *Rz2*. Due to the long use of *Rz1* as a single resistance gene, resistance-breaking populations have been developed, making *Rz2* of particular importance for future control. *Rz2* has been identified as a classical R gene belonging to the group of coiled-coil nucleotide-binding site-leucine-rich repeat (CC-NBS-LRR) proteins (Capistrano-Gossmann et al., 2017). Using a transient expression system in *Nicotiana benthamiana*, we previously showed that the transport protein triple gene block protein 1 (TGB1) of BNYVV represents the avirulence gene of *Rz2* (Wetzel et al., 2021). When coexpressed with *Rz2*, a hypersensitivity reaction (HR) with subsequent cell death can be observed. This HR response also occurred when either the TGB1 protein of the closely related beet soil-borne mosaic virus (BSBMV) or the TGB1 protein of the unrelated pomovirus beet soil-borne virus (BSBV) was inoculated in coexpression with *Rz2*. In further studies, the interaction of *Rz2* with the TGB1 proteins of different viruses should be characterized in more detail. First, the HR assay showed that the sugar beet colonizing beet virus Q (BVQ) from the genus *Pomovirus* is also recognized by *Rz2*. An HR response could be detected even when the TGB1 proteins were expressed by two viruses for which sugar beet is not a host plant. These included potato mop-top virus (PTMV, genus *Pomovirus*) and barley stripe mosaic virus (BSMV, genus *Hordeivirus*). The TGB1 proteins of the virus species recognized by *Rz2* show sequence homology only in the domains (I-VI) of the NTPase/helicase. Therefore, this region could be responsible for cross-species recognition. With the help of deletion mutants, the recognition position could be restricted to the two domains V and VI. Even highly truncated TGB1 mutants containing only domains V and VI of the NTPase/helicase triggered an HR response. Thus, it can be concluded that a highly conserved region in the TGB1 protein is essential for the HR response and cross-species recognition by *Rz2* seems possible.

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Capistrano-Gossmann, G. G., Ries, D., Holtgräwe, D., Minoche, A., Kraft, T., Frerichmann, S. L., ... & Kopisch-Obuch, F. J. (2017). Crop wild relative populations of Beta vulgaris allow direct mapping of agronomically important genes. *Nature communications* 8, 1-8.

Wetzel, V., Willlems, G., Darracq, A., Galein, Y., Liebe, S., & Varrelmann, M. (2021). The Beta vulgaris-derived resistance gene Rz2 confers broad-spectrum resistance against soilborne sugar beet-infecting viruses from different families by recognizing triple gene block protein 1. *Molecular Plant Pathology* 22, 829-842.

Manipulating betalain biosynthesis using beet mosaic virus mediated expression of BvMYB1 transcription factor allows visual virus tracking in *Beta vulgaris*

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European sugar beet growers are facing the re-emerging Virus yellows (VY) disease, which is caused by a complex of different viruses including beet mosaic virus (BtMV), genus *Potyvirus*. As a result of VY spreading throughout Europe, breeders desire natural plant resistance and robust biotests for fast and reliable identification. Virus induced symptoms in varying genotypes do not always correlate with the replication level and frequently inhomogeneous tissue colonisation over time. This often requires extensive labor for a final evaluation of potential resistance/tolerance in breeding material.

Bedoya et al. (2012) showed that the heterologues expression of the Rosea1 transcription factor from *Antirrhinum majus* using tobacco etch virus resulted in an induction of anthocyanin biosynthesis, which enables visual virus tracking in *Nicotiana benthamiana*. However, members of the order Caryophyllales produce another red pigment class the betalains, however, their biosynthesis is differentially regulated (Polturak & Aharoni 2018). Here we report the first construction of a BtMV cDNA full-length clone for generation of infectious *in vivo* transcripts and an agrobacterium mediated inoculation with 100 % efficacy in *Beta vulgaris* and *N. benthamiana*. To allow proper release from the polyprotein, the *B. vulgaris* BvMYB1 transcription factor was introduced inbetween P1 and HC-Pro of BtMV with a C-terminal NIa/NIb viral protease recognition site (BtMV-BvMYB). Infecting *B. vulgaris* with BtMV-BvMYB led to strong betalain formation in virus colonized tissue, enabling a visual, non-destructive infection tracking by the produced red pigments. Expression of betalain biosynthesis genes and the viral load were compared by RT-qPCR in beet leaves and showed a high correlation. Even with a delay of approximately two days in symptom/pigment development and a lower virus titer compared to wildtype BtMV, this molecular tool can be beneficial for a better understanding of virus-host interaction in the beet pathosystem and has strong potential for use in resistance breeding allowing high throughput screens.

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Co-infection with other sugar beet viruses modifies the transmission and localization of beet yellows virus

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Abstract

Viral multi-infection is a very common phenomenon in plants. Sugar beet is a crop frequently infected by several viruses, Some of the viruses infecting beet (the closterovirus BYV and the poleroviruses BChV&BMYV) are restricted to phloem cells while a potyvirus (BtMV), invades all cells of the plant. The four viruses are very frequently present in the field either as single or multi-infections. BYV is the most devastating sugar beet virus in France. The objective of this research was to investigate how BYV co-infections with up to three viruses change transmission and virus localization. First, we looked for and optimized a detection method to localize all four sugar beet viruses simultaneously within co-infected tissues and cells. The SABER fluorescent in situ hybridization technique was selected because of its capacity to detect multiple targets in the same microscopy section. With this technique we started first with BYV/BChV co-infection that is most observed in the field. Our results showed co-localization of the two viruses in the same phloem cells. This correlated with a higher transmission of BYV and a lower one of BChV. Also co-infection of BYV&BtMV resulted in co-localization of the two viruses in the same cells but this correlated with lower transmission of BYV. Interestingly, co-infection of BYV with the other polerovirus, BMYV, resulted in mutual cellular exclusion. The transmission experiments for this combination are in progress. In the last studied combination, BMYV, BYV & BChV triple infection, the presence of BMYV caused the sequestration of BYV & BChV in different phloem cells. This triple infection had no major effect on BYV transmission. Taken together, depending on the combination the co-infection has different impact on virus transmission and the localization. As future perspectives we will investigate the mechanisms behind the cellular exclusion or co-localization and how they impact transmission.

Host range, molecular and ultrastructural analyses of Asparagus virus 1 isolated from Asparagus officinalis L.

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Asparagus is not only a popular vegetable worldwide but of great nutritional value. Being a perennial, it is exposed to pathogens, e.g. fungi and aphids including the viruses they transmit. Asparagus virus 1 (AV-1) causes asymptomatic infections. When present with other viruses or fungi, AV-1 infection results in significant yield and quality loss (Hein 1963, Kegler et al. 1991, Elmer et al. 1996, Knaflewski et al. 2008, Lantos et al. 2018). In a survey on AV-1 diversity in asparagus, 474 samples were collected from asparagus growing regions in Germany and selected European as well as American countries. Overall 474 samples were analyzed for Asparagus virus 1 (AV-1) using DAS-ELISA and 19 AV-1 isolates were further characterized. For the first time the ultrastructure of AV-1 infection in asparagus has been revealed and could be compared with the one in indicator plants. The cylindrical inclusion protein (CI), a core factor in viral replication, localized within the cytoplasm and in systemic infections adjacent to plasmodesmata. The majority of isolates referred to pathotype I (PI). These triggered a hypersensitive resistance in Chenopodium spp. but did not infect Nicotiana spp. Only PII and PIII infected *N. benthamiana* systemically but differed in their pathogenicity when transmitted to Chenopodium spp. The newly identified PIII generated amorphous inclusion bodies and degraded chloroplasts during systemic infection but not in local lesions of infected Chenopodium spp. Phylogeny of the coat protein region recognized two clusters, which did not overlap with the CI-associated grouping of pathotypes. These results provide evidence for ongoing modular evolution of AV-1.

Comparison of the prevalence of ASaV-infected flowering ash (*F. ornus*) trees in two Germany Metropoles

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Viral diseases play a particular role in tree health as predisposing factors (Büttner et al, 2013, 2023). Therefore, the virus infection could counteract trees resilience, especially in cities with anticipated extreme climate conditions. Flowering ash (*Fraxinus ornus L.*) infected by the ash shoestring-associated virus (ASaV) develop symptoms like chlorotic ringspots, mottle and leaf deformation such as curling and shoestring (Gaskin et al., 2021). The tree species is considered as a potential "climate tree" in urban environment. The prevalence of ASaV in this tree species, which is increasingly planted in German cities, should be assessed in the cities of Hamburg and Berlin. In the vegetation periods 2019 and 2020, a survey on the occurrence of ASaV-associated symptoms was carried out in selected street sites of Hamburg considering 50 % of the 466 flowering ash trees planted in the city state. The trees were visually inspected twice per year. A selection of symptomatic and symptomless leaves was sampled and tested for an ASaV-infection by virus-specific RT-PCR.

In 2021, we examined 65% of the 1150 flowering ash trees grown in Berlin for ASaV symptoms. Visual scoring of the trees was followed by sampling of 82 symptomtic leaves. Additionally, 85 samples without virus suspected symptoms and 32 with discoloration and deformation atypical to an ASaV-infection, and 14 samples with mild ASaV-symptoms were taken and tested by ASaV-specific RT-PCR. The occurrence and distribution of ASaV-infected trees in these two cities make aware that viruses have to be listed as a considerable member of potential pathogens in trees.

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Environmental and genetic drift of the wheat-polymyxa-furovirus interaction

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The furoviruses Soil-borne wheat mosaic virus (SBWMV) and soil-borne cereal mosaic virus (SBCMV) are some of the most economically devastating cereal viruses in Germany. These viruses are transmitted by the soil-borne plasmodiophorid *Polymyxa graminis* (*P. graminis*) to roots of host plants. Currently, the only reliable method to control the viruses relies on planting of resistant cultivars carrying one or both of the two known resistance genes *Sbm1* and *Sbm2*. Both of these genes are described as translocation resistance, hampering furoviruses systemic movement to the upper plant parts. However, the influence of environemental factors (temperature, watering, fertilization) on furovirus infection and resistance stability remains poorly understood. To better estimate the importance of climate factors on the spread of SBWMV/SBCMV and on the efficiency of resistance, thirteen cultivars, differing in their grade of resistance, were sawn in infected soil. The plants were cultivated in controlled conditions under eight different climatic conditions, differing in temperature, watering and fertilization. Five replicates of eight plants per genotype and per condition, representing a total of 4 200 plants were tested by serological and molecular methods for the presence and titer of furovirus. The content of *P. graminis* was investigated using real-time RT-PCR. Our results indicate that i) both, furovirus infection and translocation rates are influenced by environmental conditions, genetics and the interactions between them, ii) only the environment influences *P. graminis* RNA content. To further understand wheat reaction to *P. graminis* and furovirus infection, roots of a susceptible and two resistant cultivars were harvested after ten days and four months of cultivation in different soils (autoclaved, containing virus free *P. graminis*, with viruliferous *P. graminis*). The transcriptome was then determined via massive analysis of cDNA ends (MACE). This work paves the way in furovirus epidemiology and pre-breeding to optimize their control in the field.

Manipulation of the plant RNA splicing machinery by geminiviruses

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Geminiviruses are a large family of plant DNA viruses causing serious problems for agriculture worldwide. They rely heavily on the host molecular machinery for replication due to their small genome size and limited coding capacity. We hypothesize that they may also take advantage of the plant RNA processing mechanisms, such as alternative splicing (AS), to maximize their transcriptome and proteome diversity. Indeed, we have found that the geminiviral replication-associated proteins (Rep) from two geminiviruses associate with the splicing machinery of the model host *Nicotiana benthamiana*, and that a functional spliceosome is required for a successful viral infection. In addition, AS of viral transcripts has been uncovered in tomato yellow leaf curl virus, leading to several new protein isoforms. Preliminary characterization of these spliced variants shows substantial differences in their subcellular localization compared to the unspliced proteins, suggesting putative novel functions. Furthermore, Rep spliced variants, lacking the oligomerization domain required for Rep role in initiating viral replication, are able to repress the Rep promoter, and may thus be needed for the later phase of the viral cycle. Finally, drastic changes in the plant AS landscape can be observed upon geminiviral infection, even if the biological relevance of this alteration is still unclear. Our data indicate the importance of splicing for the geminiviral infection, highlighting an additional battleground at the interface of plant-geminivirus interactions.

Evidence for the splicing of complementary-sense transcripts of Beet curly top Iran virus

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Beet curly top Iran virus (BCTIV) is the type member of the genus Becurtovirus (Family Geminiviridae) with a circular single-strand DNA genome. The BCTIV genome contains five genes including C1 and C2 on the complementary-sense DNA strand and V1, V2 and V3 on the virion-sense DNA strand. The nucleotide sequences of both C1 and C2 genes are similar to their cognate genes in the genus Mastrevirus which encode proteins responsible for virus replication. The C1 gene in monopartite geminiviruses encodes the replication-associated protein (Rep), but in mastreviruses, the full-length Rep is translated from the spliced transcripts of C1 and C2 genes. However, the splicing feature in BCTIV and the role of the spliced products are unknown. This study presents characterization of complementary sense transcripts of BCTIV and the viral factors in directing the pathogenicity and hypersensitive response (HR) in Nicotiana benthamiana plants. In both local and systemic infection, splicing of the complementary transcripts of BCTIV was observed. Notably, a small number (8.3 %) of transcripts were spliced to produce Rep (C1:C2) transcripts after deletion of 155 nt (position 1892-2046 from BCTIV). In addition, transcript mapping to the BCTIV genome confirmed splicing in C1:C2 genes in infected plants. Expression of BCTIV genes in N. benthamiana using the tobacco rattle virus (TRV) based vector showed that Rep together with C1 are the main pathogenicity factors which cause typical viral leaf curling symptoms. In addition, the V2 caused a mild leaf curling, thickening and asymmetric leaves. Transient expression of individual viral genes showed that both the C1 and Rep trigger a Hypersensitive resistance (HR) response in *N. benthamiana*. It is concluded that Rep and C1 are the main pathogenicity factors that also trigger HR response in plants.

Analyzing splicing as novel strategy of bipartite begomoviruses to increase their coding capacity

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Stress affects plant growth as well as their development. Plant viruses are biotic stresses that pose a significant threat to food supply with overpopulation and climate change. In eukaryotic genes, protein-coding sequences are often interrupted by introns, which are removed during pre-mRNA splicing. The exons are ligated to form the matured mRNA. The process takes place in spliceosomes. Plants use pre-mRNA splicing to respond to negative environmental conditions. Through splicing, they regulate the expression of stress genes and reprogram intracellular regulatory networks. It is becoming increasingly clear that various stress factors influence alternative splicing of stress genes in plants. Plant viruses such as geminiviruses have limited coding capacity due to small genome sizes and are dependent on host cell machinery. In the geminiviruses Mastrevirus, Becurtovirus and Grablovirus, Rep is spliced.

In the present work, the bipartite begomovirus AbMV was examined by PCR analysis for splicing to increase coding capacity. A deleted CP ORF (site: 659-694 nt; size: 36 bp) and possibly a second DNA circle were detected when examined for a deleted Rep ORF (site: 2048-2206 nt; size: 159 bp). This second DNA circle, which could have arisen by DNA repair with mRNA, lacks exactly the expected intron. For the monopartite begomovirus TYLCV, the truncated CP (site: 634-745 nt; size: 112 bp) detected by Rosa Lozano-Duran's group could be confirmed, whereas the deleted Rep (site: 2049-2207 nt; size: 159 bp) could not.

keynote

Lost in translation? The long-distance travel of messenger RNAs

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Apart from small sugars, peptides and hormones, also large proteins and RNAs are allocated via the vasculature to distant tissues. This includes all major classes of endogenous RNAs such as small si/miRNAs, transfer RNAs, and full-length protein encoding messenger RNAs (mRNAs). Recent advances in the analysis of the mobile transcriptomes from grafted Arabidopsis plants indicate that hundreds of mRNA transcripts move along the plant axis in both directions from shoot to root and from root to shoot. Single cell and tissue wide transcriptomes revealed that a surprisingly high number of mRNAs are delivered to distinct cell types and tissues such as flowers, leaves and/or roots. Our data indicate that that transported mRNAs are translated in recipient cells and that their transport does not depend on expression levels or stability. Rather a selective mRNA transport mechanism seems to be employed involving RNA-binding proteins, distinct RNA motifs and secondary m^5C base modifications. In line, the mobile mRNA transcriptome changes depending on developmental stages and growth conditions and, as indicated with some graft-mobile mRNAs, is required for normal growth. More recently, we have shown that Cas9 / gRNA root-to-shoot transport can be achieved by a tRNA-like sequence (TLS) motif(s). Such mobile Cas9-TLS / gRNA-TLS fusion transcripts move to grafted wild-type scions which form transgene free edited seeds in one generation.

A viral peptide as a trigger for the relocalization of a viral effector

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The activation of the first layer of host innate immune responses against invading microbial pathogens relies on recognition of conserved pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). Tomato yellow leaf curl virus (TYLCV), and more specifically its replication-associated protein (Rep), induces plant defense, which triggers translocation of a viral C4 protein from the plasma membrane (PM) to chloroplasts in the host cells; from this organelle, C4 suppresses salicylic acid-dependent defenses to promote the infection. However, how Rep activates plant defense is unknown. Here, we identify a novel open reading frame (ORF) embedded within that of *Rep* in the TYLCV genome, which we named Peptide Viral Gene (PVG) and encodes a predicted secreted peptide. RNA-sequencing results show that exogenous application of PVG activates plant defense responses. The TYLCV PVG is not only essential for full virulence, but also, strikingly, sufficient and required to shift the localization of the viral TYLCV C4 protein from PM to chloroplasts. Stable transgenic tomato lines expressing the non-myristoylable chloroplast-localized TYLCV $C4_{G2A}$ complement PVG deficiency in TYLCV during viral infection. This finding suggests that the main biological function of PVG is to direct C4 to chloroplasts, where it ultimately suppresses plant defense responses. Taking advantage on CRISPR-Cas9 edited *N. benthamiana* plants, we confirm that the receptor-associated kinases SOBIR1 and BAK1 are required for PVG to be perceived by the plant. In summary, our findings suggest that PVG functions as a viral PAMP mimic, possibly being perceived by yet-to-be determined receptor(s) in host plants to trigger the re-localization of a viral virulence factor to a second site of action.

A novel assay demonstrates a possible mode of action for Beet Curly Top Iran Virus V2 as a suppressor of transcriptional gene silencing

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Suppression of transcriptional gene silencing (TGS) is imperative in allowing proliferation of DNA viruses in plants.[1] TGS suppression can inhibit the initiation or the maintenance of methylated sites or it may involve active demethylation of the target sites. In order to assess the exact pathway that viral TGS suppressor proteins interfere with, we developed transgenic TGS lines of *Nicotiana benthamiana*, which contain a quantifiable trigger to suppress GFP expression (16C-qRdDM) and use an epigenetically modified *N.benthamiana*, which has enriched CG methylation in the promoter of GFP (16C-metCG). Using 16C-qRdDM, 16C-metCG, and previously published 16C-TGS lines[2] we addressed the role of Beet Curly Top Iran Virus (BCTIV) V2 protein in the suppression of TGS. We found that V2 can suppress neither the initiation of silencing in the presence of a silencing trigger nor demethylate the promoters actively. Rather, V2 could repress the maintenance of methylation in the newly developing tissues. Bisulfite analysis points out that the V2 expression via Tobacco Rattle Virus (TRV) vectors lead to the accumulation of fully unmethylated promoter sequences, which in turn increase GFP expression in mRNA and at the protein level. Next, we focus on the changes in the chromatin structure induced by the ectopic expression of the BCTIV V2 protein. Essentially, in this study, we identified BCTIV V2 as a suppressor of TGS and narrowed down the possible modes of action, using a system containing novel TGS reporter lines, applicable to future research of viral TGS suppressors.

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A plant virus causes symptoms through the deployment of a host-mimicking protein domain to attract the insect vector

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Viruses are obligate intracellular parasites with limited proteomes that heavily rely on the cell molecular machinery for their multiplication and spread. Plant viruses frequently cause symptoms through interference with host developmental programs. Despite the agricultural relevance of symptom development in virus-infected crops, the molecular mechanisms underlying these viral effects remain elusive. Here, we show that the symptoms triggered by tomato yellow leaf curl virus (TYLCV) depend on the physical interaction between the host-mimicking domain of a virus-encoded protein, C4, and a plant-specific family of RCC1-like domain-containing (RLD) proteins. C4 outcompetes endogenous interactors of RLDs, disrupting RLD function in the regulation of endomembrane trafficking and auxin polar transport, ultimately leading to the developmental alterations recognized as symptoms of the viral infection. Importantly, symptoms do not have a detectable effect on the performance of the virus in the plant host, but they serve as attractants for the viral insect vector, the whitefly *Bemisia tabaci*, hence promoting pathogen spread. Our work uncovers the molecular underpinnings of the viral manipulation that leads to symptom development in the TYLCV-tomato pathosystem, and suggests that symptoms have evolved as a strategy to promote viral transmission by the insect vector. Given that most plant viruses are insect-transmitted, the principles described here might have broad applicability to crop-virus interactions.

Development of serological test methods for the detection of novel emaraviruses in oak and ash trees

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In recent years, numerous novel emaraviruses have been described in important deciduous trees species of forests and urban green. This group of plant pathogenic viruses, recently summarized by Rehanek et al. (2022) in a review, is distributed worldwide and has a segmented negative-strand RNA genome It comprises a core of four genome segments, with each RNA encoding a virus-specific protein (Rehanek et al. 2022). In common oak (*Quercus robur* L.) and common ash (*Fraxinus excelsior* L.), novel emaraviruses associated with characteristic virus-suspected leaf symptoms were identified by high-throughput sequencing (HTS). The common oak ringspot-associated virus (CORaV) and the ash shoestring-associated virus (ASaV) represent distinct species within the genus *Emaravirus* and are widespread in different European countries (Rehanek et al. 2021; Gaskin et al. 2021; Svanella-Dumas et al. 2022). While RT-PCR is established as the standard diagnostic method for the detection of most emaraviruses, serological detection methods for forest tree-infecting viruses are rarely available. We aim to develop diagnostic ELISAs for the CORaV and ASaV detection in the economically important tree species oak and ash. The RNA 3 encoded proteins were heterologously expressed and purified for immunisation in order to obtain polyclonal antibodies against the viral nucleocapsid proteins. First results are presented.

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Screening German chickpeas for different viral infections

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Legumes play a crucial role in modern agriculture as they serve as a valuable source of protein for human and animal consumption. Apart from their nutritional benefits, they also provide environmental advantages due to their nitrogen-fixing abilities. However, cultivated legumes are susceptible to various viral infections, which can cause significant losses in yield and quality. Until 2020, the International Committee on Taxonomy of Viruses has identified 168 viruses from 39 genera and 16 families that can affect leguminous crops, such as chickpea, pea, soybean and alfalfa. Chickpea, which is adapted to drought conditions, is an attractive option for crop rotation in organic farming and is gaining popularity in Germany. Therefore, this study aims to investigate the impact of legume-infecting viruses on chickpea crops. In 2022, we collected sample material from symptomatic plants at two locations and analyzed it for the presence of circular (ss) DNA viruses, such as pea necrotic yellow dwarf virus (PNYDV), using a combination of rolling circle amplification (RCA) and downstream restriction fragment length pattern (RFLP) analysis. Furthermore, we conducted a reverse transcription-PCR (RT-PCR) to detect different legume-infecting RNA viruses. Our analysis confirmed the presence of PNYDV and various RNA-viruses in the sample material, including turnip yellows virus (TuYV) and pea enation mosaic virus-1 (PEMV-1). Here, we will show representative images of infected field samples and provide a summary of the RCA/RFLP and RT-PCR results. We will also discuss the potential threat to chickpea production and the use of resistant cultivars in the future.

Construction of a turnip yellows virus (TuYV) cDNA full-length clone of an isolate originating from sugar beet to study its host plant spectrum

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Turnip yellows virus (TuYV) is a predominantly *Brassicaceae* infecting isolate in the genus *Polerovirus* of the family *Solemoviridae*. It has an enormous economically impact on oilseed rape, lettuce and other field crops including members of the *Fabaceae*. Sugar beet is considered a non-host. In a recent European-wide virus yellows monitoring in sugar beet (Hossain et al., 2021), a polerovirus isolate which could be assigned to the species TuYV was identified by deep sequencing in a leaf sample from the Netherlands. Recently, reports occurred about the detection of additional sugar beet infecting TuYV isolates in the Netherlands and Sweden. By RACE analysis of the genomic RNA, the complete sequence could be determined. The identified TuYV isolate shares overall nucleotide sequence identity of 98 % with TuYV isolated from pea (OK030772). Using the complete genome sequence, a full-length cDNA clone under control of the cauliflower mosaic virus 35S promoter was constructed by Gibson assembly. Agrobacterium-mediated leaf infiltration under greenhouse conditions revealed clone infectivity in *Nicotiana benthamiana*, *Pisum sativum* and *Brassica napus*, which was unexpected because sequence alignments with Brassica-infecting TuYV isolates demonstrated clear differences in P1, which is putatively responsible for host specificity. So far, it has not been shown that the clone is capable of infecting sugar beet. Results of an aphid transmission assay from *B. napus* infected plants to healthy sugar beet plants are pending. The cDNA clone and knowledge of its complete genomic sequence can be used as a beneficial molecular tool for a better understanding of the virus-host interaction. In the risk of further spread of TuYV in sugar beet cultivation, it can serve as basis to develop solutions for disease control and to support breeders to identify resistance sources against this putatively emerging virus in sugar beet.

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Tomato brown rugose fruit virus in the Netherlands: Epidemiological insights with Nextstrain

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In the Netherlands, tomato brown rugose fruit virus (ToBRFV) was first identified in tomato crops in 2019. Since then, the National Plant Protection Organization (NPPO-NL) is performing surveys to track and trace this regulated virus aiming for its eradication. According to the EU implementing regulation, samples are tested with real-time RT-PCR for virus detection and confirmation. Additionally, to gain more insight in the epidemiology of this virus, for samples with sufficiently high virus concentration, whole genomes are assembled based on Illumina RNA sequencing data. Whole-genome phylogenetics was integrated with meta-data such as host, variety, seed batch, geographic location, but found no associations. The outcome indicates that multiple introductions may have accounted for the outbreaks in the Netherlands. Furthermore, the analyses suggests that the virus was probably already present in the Netherlands in 2018 or 2017 and had been introduced at least four times (van de Vossenberg et al., 2020, https://nextstrain.nrcnvwa.nl/ToBRFV). Thanks to strict hygiene measures, some infested growers managed to eradicate the virus during crop rotation. Growers that did not succeed, remained infested with the same virus sequence type, suggesting elimination was unsuccessful in these cases. Intriguingly, in 2021 this pattern changed, when several re-infestations concerned a common novel virus sequence type, indicating a shared source.

NPPO-NL maintains a publicly available interactive ToBRFV Nextstrain webpage (https://nextstrain.nrcnvwa.nl/ToBRFV) displaying data from the tracing research supplemented with genome sequences that are either retrieved from NCBI GenBank or kindly shared by international partners. This dataset facilitates a better understanding of the global diversity and spread of ToBRFV.

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Tomato brown rugose fruit virus contaminated clothing of greenhouse employees is a challenge for cleaning

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The highly virulent and mechanically easily transmissible *Tomato brown rugose fruit virus* (ToBRFV) has already been detected in large parts of the world and caused massive economic damage in commercial greenhouse tomato production. Transmission of ToBRFV in greenhouses results particularly through humans during cultivation measures such as pruning or harvesting activities (González Concha et al. 2021). In this context, the risk of spread via contaminated clothing must be highlighted, which has not been addressed in recent studies. In this study, we evaluated the efficacy of several detergents and one disinfectant in terms of their cleaning capacity on heavy ToBRFV-contaminated clothing in bioassays (Nourinejhad et al. 2022, 2023). We figured out, that household detergents cannot effectively remove ToBRFV from the fabric while at the same time the resulting cleaning solutions are highly contaminated. In contrast, agricultural detergents and an approved disinfectant resulted in almost complete removal or inactivation of ToBRFV both on the fabric and in the cleaning solutions. Contaminated cleaning solutions could be decontaminated by the additional application of MENNO Florades (Ehlers et al. 2022).

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Potential risk of Tomato brown rugose fruit virus carry-over by surfaces in a commercial tomato greenhouse

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The economically important tobamovirus *Tomato brown rugose fruit virus* (ToBRFV) has already emerged in numerous important tomatoes producing countries since its first appearance in Israel in 2014 (Caruso et al. 2022). In October 2021, regulation (EU) 2021/1809, softened the emergency measures for ToBRFV in Europe to allow fruit-producing tomato businesses to continue cultivating until the end of the growing season despite a ToBRFV detection and to mitigate the economic consequences. But, what are the consequences of continuing to grow on infested farms for the spread of this stable and mechanically easily transmitted virus. What is the role of humans in the spread of the virus on the farm and, if applicable, what routes does it take (Ehlers et al. 2022a, b)?

In this context, numerous samples were taken on a ToBRFV infected production site in Germany in 2022. Various surfaces of different areas including cultivation and packing as well as common and private rooms of workers were sampled. These swap samples were analyzed in bioassays (Nourinejhad et al. 2022) and ELISA for a contamination with ToBRFV. With knowledge of the areas with increased or even high ToBRFV contamination, a company-specific hygiene concept can be developed. Only with a holistic approach, which includes a comprehensive hygiene plan, will it be possible to greatly slow down or, in the best case, even prevent the spread of ToBRFV on the horticultural farm.

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Virusdiagnostik für den amtlichen Pflanzenschutzdienst in Brandenburg

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Im Referat L3 des Landesamtes für Ländliche Entwicklung, Landwirtschaft und Flurneuordnung (LELF) werden pflanzengesundheitliche Untersuchungen für den amtlichen Pflanzenschutzdienst sowie für die Saatenanerkennungsstelle des Landes Brandenburg durchgeführt. Das beinhaltet Diagnosen von biotischen Schadursachen geschädigter Kulturpflanzen im Rahmen von Verdachtsproben, Erhebungen, Monitorings und Schaderregerüberwachungen sowie die Attestierung der Befallsfreiheit bei pflanzlicher Handels- und Vermehrungsware.

Die Präsentation beinhaltet einen Überblick über Probenart, Probenumfänge und Prüfverfahren der pflanzenvirologischen Diagnostik im Land Brandenburg.Weiterhin wird die besondere Situation der Virustestung von Pflanzkartoffeln in Brandenburg im Jahr 2022 dargestellt.

Online flash talks

Cassava brown streak HAM1 gene and its role in the resistant response in cassava

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Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) cause the cassava brown streak disease, which leads to necrosis of the roots and, thus, the destruction of the edible root tubers. We have identified resistance against these severe pathogens in South American germplasm lines, which acts against both virus species or targets only one. In resistant plants, virus replication is blocked, the virus is confined to the phloem companion cells, and there is no translocation from phloem cells to the neighboring parenchyma tissues.

By using infectious cDNA clones of the viruses, GFP-labeled viruses, and recombinant exchange mutants of the viruses, the infection processes in cassava and *N. benthamiana* were followed to gain insight into viral and host genes associated with resistance responses.

The unique HAM1 gene was required for cassava infection; however, when removed from the genome, the virus still infects *N. benthamiana*, but in contrast to the wild-type CBSV, there were no necrotic lesions visible in inoculated and systemically infected leaves. We exchanged CBSV HAM1 with HAM1 of UCBSV (CBSVΔHAMKe), resulting in infections that reduced virus titers and attenuated symptoms in cassava. In contrast, replacing HAM1 of UCBSV with the respective gene of CBSV (UCBSVΔHAMMo) enhanced symptom expression and virus replication, and plant invasion dramatically. Furthermore, the symptoms caused by HAM1 recombinant viruses resembled those of the wild-type virus from which Ham1 was extracted. Thus, HAM1 defines symptom expression and virus replication and thus is a virulence gene of the virus.

We used RNAscope® *in situ* hybridization to localize virus RNA and HAM1 recombinant cDNA clones in resistant cassava lines showing differential responses to CBSV and UCBSV. CBSV was barely detectable in phloem companion cells, while UCBSV was abundantly present in parenchyma cells of the differential host. Infections of the same cassava lines with the CBSV HAM1 recombinant UCBSV (UCBSVΔHAMMo) resulted in a resistance response. There was no replication, and the recombinant virus was restricted to phloem companion cells only. This highlights the function of HAM1 in virus translocation and movement.

Dynamic subcellular distribution of begomoviral nuclear shuttle and movement proteins

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The bipartite geminivirus Abutilon mosaic virus (AbMV) encodes on its DNA-B component a nuclear shuttle protein (NSP), and a movement protein (MP) which cooperatively accomplish viral DNA transport through the plant. Subcellular distribution patterns of fluorescent protein-tagged NSP and MP were tracked in Nicotiana benthamiana leaves in presence or absence of an AbMV infection using light microscopy. NSP was located within the nucleus and associated with early endosomes in the presence of MP. MP appeared at the plasma membrane, plasmodesmata and in motile vesicles, trafficking along the endoplasmic reticulum in an actin-dependent manner. MP and NSP did not co-localize and employed separate cellular pathways. Correspondingly, Förster resonance energy transfer analysis did not support physical interaction between NSP and MP. Time lapse movies illustrate the cellular dynamics of both proteins on their way around the nucleus and to the cell periphery and provide a first hint for the nuclear egress of NSP complexes.

Emerging multifactorial complexity at the geminivirus-host interface

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Geminiviruses, which are circular single-strand DNA (ssDNA) plant viruses, are responsible for enormous crop loss worldwide. To complete their infection cycle in the host cell, geminiviruses need to effectively deploy their own proteins to enable viral replication and spread. Due to size constraints, the coding capacity of the viral genome is limited; how the few virus-encoded proteins lead to significant host cell reprogramming remains unclear. Although the geminivirus tomato yellow leaf curl virus (TYLCV) was believed to encode 6 proteins, recent results indicate that additional viral proteins exist (Gong and Tan *et al.*, 2021; Zhao *et al.*, 2022): on one hand, the TYLCV genome encodes small open reading frames (ORFs) that could give rise to novel proteins; on the other hand, additional protein variants are generated through alternative splicing. Therefore, we propose that the entire proteome of geminiviruses must be re-defined, and the function of each viral protein needs to be investigated in the context of the viral infection. We combine genomic, interactomic, and functional approaches to analyze three geminiviruses from different genera, TYLCV (gen. *Begomovirus*), beet curly top virus (BCTV, gen. *Curtovirus*) and chickpea chlorotic dwarf virus (CpCDV, gen. *Mastrevirus*), with the aim to infer a comprehensive map of the geminivirus/host cell intersection. Recent results on the identification of novel ORFs and intra-viral proteome connectivity in these three viruses will be presented.

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Investigating the role of the chromatin regulator LHP1 in defense against geminiviruses and its interplay with the geminivirus-encoded C3 protein

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Geminiviruses have emerged as devastating pathogens causing huge losses in crops worldwide. Geminiviruses replicate their circular single-stranded (ss) DNA genomes in the nuclei of plant cells through double-stranded (ds) DNA intermediates that are wrapped around histones, forming minichromosomes (Hanley-Bowdoin et al., 2013). The geminiviral chromatin is subjected to epigenetic modifications that have been implicated in the regulation of viral pathogenesis (Zarreen and Chakraborty, 2020). However, our understanding of the plant-geminivirus epigenetic battlefield is still limited. C3 is an enhancer of viral replication with a yet-to-be-described essential role for the infection (Wu et al., 2021). Using C3 from the geminivirus tomato yellow leaf curl virus (TYLCV) as a bait against a cDNA library from infected tomato plants (Rosas-Diaz et al., 2018), the LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) protein, a Polycomb protein binding H3K27me3 with a function in transcriptional repression, was identified and confirmed as a direct interactor of the TYLCV C3 protein. Tobacco rattle virus (TRV)-mediated virus-induced gene silencing experiments show that LHP1 plays an anti-geminiviral role in tomato and *N. benthamiana*. Strikingly, transcriptional analysis revealed that C3 largely changes the transcriptional landscape of the host in the context of the TYLCV infection; whether these changes are linked to its interaction with LHP1 requires further investigation. This study will expand our understanding of the epigenetic changes underlying the outcome of geminivirus-plant interactions, and provide insight into the role of LHP1 in the ongoing arms race between plant defense and viral counterdefense.

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Unravelling the molecular mechanisms underlying the replication of geminiviruses

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Geminiviruses are a family of plant viruses characterized by twin icosahedral capsids and circular, single-stranded DNA genomes. They cause devastating diseases in crops around the world. Replication of geminiviruses is the first step of the viral cycle. We have recently shown that DNA polymerase α and δ are required for viral replication. However, the composition of the viral replisome remains mostly elusive. The viral replication initiation protein (Rep) is highly conserved among geminiviruses, and is the only viral protein essential for replication. Here, we use Rep from tomato yellow leaf curl virus (TYLCV) and Abutilon mosaic virus (AbMV) as a bait to capture host factors involved in viral DNA replication in *Nicotiana benthamiana* via TurboID-based proximity labelling (PL) followed by mass spectrometry (MS) analysis. Functional validation of the PL-MS data has unveiled splicing as a process required for viral replication. In addition, we have identified novel factors required for geminiviral replication, including novel components of the viral replisome, e.g., polymerase delta, replication factor C and PCNA. Together, these results shed light on how geminiviruses repurpose the eukaryotic DNA replication machinery to perform rolling-circle replication.

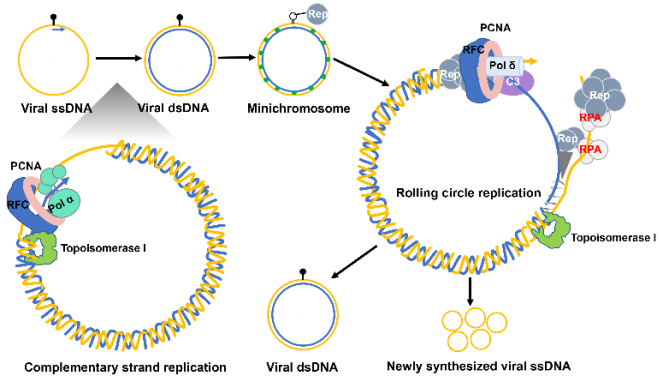

Figure 1. Hypothetical model. Schematic representation of how geminiviruses re-purpose host factors to perform rolling circle replication.

Uncovering novel virulence strategies deployed by tomato yellow leaf curl virus

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Geminiviruses are a family of circular single-stranded DNA viruses, with reduced genome size and limited coding capacity. The 4-8 proteins geminiviruses encode are multifunctional and cooperatively modulate the host molecular machinery to fulfill the viral life cycle. How viruses utilize a handful of proteins to reprogram host cells during viral infection remains a long-standing enigma in virology. In this work, we explored viral virulence strategies using tomato yellow leaf curl virus (TYLCV) as a model geminiviral species. On the one hand, we showed that the six canonical proteins encoded by TYLCV physically associate with one another in an intricate network. Our results further prove that these interactions can modify the subcellular localization of the viral proteins involved and that co-expressed interacting viral proteins can exert novel biological functions in planta that go beyond the sum of their individual functions. On the other hand, through bioinformatic prediction, we found that geminiviral genomes contain additional conserved open reading frames (ORFs). We selected 6 ORFs (ORF1-6) based on their size and prevalence in TYLCV and related genomes and demonstrated that some of these novel ORFs are expressed during infection and that the encoded proteins contain predicted domains and display specific subcellular localizations. Remarkably, the proteins encoded by four of these ORFs could be detected by affinity purification followed by mass spectrometry (AP-MS) experiments from TYLCV-infected Nicotiana benthamiana cells expressing GFP-tagged versions of the viral proteins. We proved that the protein encoded by the largest of these new ORFs (ORF6) is required for full viral infection and acts as an RNA silencing suppressor. Taken together, our results suggest that the geminiviral proteome and its functional landscape can be largely expanded. Getting a comprehensive overview of the molecular plant-geminivirus interactions will require the detailed study of small ORFs so far neglected and of the function of viral proteins in combination with other elements within the viral proteome in the context of the infection.